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Computational Characterization and Immunoinformatic Analysis of Protein LipL21 From *Leptospira interrogans* serovar autumnalis

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ABSTRACT

LipL21 is a novel surface exposed lipoprotein which is expressed during infection and conserved among pathogenic species of *Leptospira*. Lipoproteins have been shown to play a key role in adhesion to host cells, modulation of inflammatory processes and translocation of virulence factors into the host cells. In this study, bioinformatics approach has been adopted to explore the properties of LipL21. Protein modeling of LipL21was performed to understand the insights of the molecular structure. The species of *Leptospira* was clustered together and form a group with highest bootstrap value. B-cell epitopes prediction of LipL21 found to be potential, so this study might be useful in creating the peptide vaccines that target the lipoprotein surface epitopes expressed during *Leptospira*.

Keywords: *Leptospira*, LipL21, hydropathicity, protein modelling, epitope, bioinformatics

INTRODUCTION

Leptospirosis has re-emerged globally as a chief zoonotic disease caused by the pathogen in tropical and subtropical regions of the world. Various animal species act as carriers for this pathogen. *Leptospira* causing a major public health problem **[1, 2]**. It is caused by different pathogenic species of *Leptospira* and is difficult to control and eradicate in tropical developing countries like India. *Leptospira* are a diverse group of spirochete bacteria classified into 13 pathogenic species and >300 serovars **[3-5]**. In human, leptospirosis suffering patients include the major symptoms children primarily show fever, vomiting, headache, diarrhea, abdominal and generalized muscle pain, whereas adults have fever, headache, anorexia, muscle pain and constipation **[6, 7]**. The severity of disease is characterized by the presence of jaundice, acute renal or hepatic failure, and pulmonary hemorrhages even leading to death **[8-10]**.

Currently available veterinarian vaccines are based on inactivated whole cell or membrane preparations of pathogenic leptospires. These types of vaccine confer protective responses through induction of antibodies against *Leptospira*l lipopolysaccharide **[11]**. However, these vaccines fail to induce long-term protection against infection and do not provide cross-protective immunity against *Leptospira* l serovars not included in the vaccine preparation. Outer membrane proteins (OMP) constitute about half the mass of bacterial outer membrane and play a significant role in adhesion, immunity, and pathogenicity. The most abundant outer membrane proteins of the *Leptospira sps*. are LipL36, LipL21 and LipL46 **[12, 13]**.

Early initiation of antimicrobial therapy may prevent some patients from progressing to more severe disease. Identification of leptospirosis in its early stages is largely a clinical diagnosis and relies on a high index of suspicion based on the patient's risk factors, exposure history, and presenting signs and symptoms. For these reasons, empirical therapy should be initiated as soon as the diagnosis of leptospirosis is suspected. Previous study evidenced the protective immunization with the protein LipL21 against leptospirosis is a potential candidate for the development of *Leptospira*l vaccine **[14]**. In continuation, the aim of the present study is the structural and functional characterization the protein LipL21 autumnalis, epitope prediction in LipL21 and genetic relatedness with other species of *Leptospira* **[15]**.

MATERIAL AND METHODS

1 Sequence retrieval

Amino acid sequence of the outer membrane protein LipL21 from *Leptospira interrogans* serovar autumnal were retrieved from UniProt KB Database (http://www.uniprot.org/uniprot/) **[16]** in FASTA format having uniport ID A0A144I2F4. The BLAST search was also performed to identify the presence of these protein in the other stains of *Leptospira*.

2 Physicochemical characterization

The physicochemical parameters of the proteins LipL21 and were calculated by Expasy's ProtParam server **[17]**, which include molecular weight, theoretical isoelectric point (pI), extinction coefficient, aliphatic index, halflife, instability index, total number of positive and negative residues, and grand average hydropathicity (GRAVY).

Functional characterization

Functional characterization of protein was performed by using SOSUI Server **[18]** to identify transmembrane regions, nature of proteins and its hydrophobicity **[19-22]**. Phylogenetic analysis of 34 sequecces of LipL21 from different species of *Leptospira* was generated by using MEGA X **[23]** based on neighbor-joining method **[24]**.

4 Protein Structural Modelling

Secondary structural analysis of the protein LipL21 was performed using GOR IV **[25]**. The 3D structulal modelling was done by Swiss – Model Workspace, a tool based on homology modelling **[26, 27]**. The generated model was visualized using Swiss- PDB viewer **[28]**, evaluated using Rampage **[29, 30]**. Energy minimized models were assessed by PROCHECK **[31]** to analyse the stereo chemical quality and residual geometry of the model by submitting the co-ordinate file in Structural Analysis and Verification Server (SAVES v5.0).

5 Computational mapping of epitopes

Linear and discontinuous B-cell epitopes of LipL21 was done from generation of three-dimensional structure. Linear B-cell epitopes were chosen with two different approaches i.e. BepiPred, ABCPred and Ellipro. ABCPred uses recurrent neural network to predict B-cell epitopes **[32]**. BepiPred-2.0 is a sequential B-cell epitope prediction tool analyse each amino acid independently and does not have a minimum or maximum number of amino acids range to predict an epitope. BepiPred-2.0 is based on hidden markov model and propensity scale method **[33]**. Discontinuous epitopes are predicted from 3-D structure by Ellipro which is an antibody epitope prediction **[34]**. The predicted epitopes were further verified by Vaxi Jen server 2.0 to identify the probality of antigen with threshold of 0.4 **[35]**. Vaxi Jen server 2.0 uses an alignment free approach for antigen prediction and works on an auto cross covariance transformation of protein sequence into uniform vectors of principal amino acid properties **[36]**.

RESULTS AND DISCUSSION

The protein sequences of LipL21 from *Leptospira interrogans* serovar autumnal was retrieved in the FASTA from UniProtKb database having UniProt ID A0A144I2F4. The BLAST search against this protein and 34 orthologous sequences of different species of *Leptospira* was performed **(Table 1)**.

The physicochemical properties **(Table 2)** have been computed which determine the fundamental properties of the protein. The values of isoelectric point (pI) of the protein sequences LipL21 indicates that it is alkaline in nature **(Table 1)**. The aliphatic index (AI), defined as the relative volume of a protein occupied by aliphatic side chains, is regarded as a positive factor for the increase of thermal stability of globular proteins.It has been observed that protein sequence LipL21 was basic in nature, the computed isolelectric point will be useful for developing buffer system when these proteins are to be purified in solution by isoelectric focusing method **[37]**. The Grand Average hydropathy (GRAVY) value for a peptide or protein is calculated as the sum of hydropathy values of all the amino acids, divided by the total number of residues present in the protein sequences **[38]**. The antibacterial or self-defense peptides which are usually highly basic may recognize the acidic phospholipids exposed on the surface of the bacterial membrane and are believed to act by disrupting the plasma membrane leading to the lysis of the cell. Extinction coefficient of protein sequences LipL21 depicts that it may be useful in the quantitative study of protein-protein and protein-ligand interactions in solution **[39]**. Further it has analyzed that Gravy Indices was found to be -0.425, this lower range of value indicated better interaction with water **[40]** **(Table 2)**. In functional characterization it has been observed that Lip21 is soluble in nature but no trans-membrane region was found tabulate in **Table 3**.

Phylogenetic Characterization and Threading Based-Epitope Mapping of *Leptospira*l Outer Membrane Lipoprotein LipL21 the branch length is considered to be statistically significant. MEGA X inferred the evolutionary tree by a Neighbour-Joining (NJ) algorithm by using a matrix of pairwise distances. In order to resolve the relationships of the sequences within each group of the constructed phylogenetic tree **(Figure 1)** was constructed with 34 sequences from different serovars **(Table 1)**. In phylogenetic analyses it has been depicted that the tree divides all the sequences into two major clusters. The first cluster further divided into two sub clusters. The analysis revealed that the protein LipL21 from *Leptospira interrogans* serovar autumnal in having closest association with *Leptospira noguchii* and *Leptospira krischneri* **[36]**. It shows the highest bootstrap value of 99, indicate that the clade is close to 100%, which reveals that all the characters in a group believed to comprise all the evolutionary descendants of a common ancestor which is rooted with different serovars and strains as the ancestral group. The phylogenetic tree has been constructed for these sequences by Neighbour-Joining method using MEGA X **(Figure 1)**.

The protein modelling was done by using secondary and tertiary structure prediction. The secondary structure indicates whether a given amino acid lies in a helix, strand or coil **[40]**. The secondary structure folding has been identified by GOR (Garnier-

Osguthorpe-Robson) method, which showed the presence of random coils in higher rangeas compared to other confirmations. The result revealed that random coils dominated among secondary structure elements. The secondary structure were predicted by using default parameters (Window width: 17, similarity threshold: 8 and number of states: 4). The conformational entropy associated with random coils significantly contributes to stabilization and protein folding.

The protein secondary structural analysis by GOR IV showed the dominance of random coil in comparison to extended strand and alpha helix i.e Random Coil<Extended Strand<Alpha Helix **(Table 4)**.

The Φ and Ψ distribution of the Ramachandran Map generated by Rampage (Assessment of the Ramachandran Plot). The red regions in the graph indicated the most allowed regions whereas the yellow regions represented allowed regions. Glycine is represented by triangles and other residues are represented by squares. The result revealed that more that 95.9% residues were in favoured regions **(Table 5)**. In the Ramachandran plot analysis, the residues were classified according to its regions in the quadrangle. The stereochemical quality of the predicted models and accuracy of the protein model was evaluated after the refinement process using Ramachandran Map calculations computed with the PROCHECK program.

3D structure features of the models were consistent with the results of their secondary structure. All these results indicated that the structural models of the LipL21 is reliable. The protein modelling was performed by using the template model PDB id: 5ok8.3.A which have 3 other biounits; a crystal structure of protein Lpp20 from *Helicobacter pylori*consisting of 175 amino acids. The tertiary structure prediction of the protein LipL21 was done by homology modelling method using SWISS-MODEL Workspace **(Figure 2)**.

Quality of generated models was evaluated by RAMPAGE program and Ramachandran plot was generated using PROCHECK. The distribution of the main chain bond lengths and bond angles were found to be within the limits for these proteins, represent a good quality of the predicted models **(Figure 3)**.

ABCPred is able to predict epitopes with approximately 66% accuracy using the recurrent neural network **[41]**. Further, the immunoinformatic analysis of antigenic peptides were done by using three different programs for epitope prediction. BepiPred-2.0 predicted B-cell epitopes at various position in LipL21 from *Leptospira interrogans serovar Autumnalis* **(Figure 4 a and b)**.

ABCPred predicts 14 overlapping and potential immunogenic regions within LipL21 **(Table 6)**. It depicted that ABCPred assign score between 0 to 1 for each epitope. If the prediction score is closer to 1, the particular prediction can be taken as epitope and if the score is closure to 0, it is not suitable for an epitope.

Three B-cell structure of LipL21 were mapped from predicted 3-D structure by using Ellipro, epitopes spans as shown in **Table 7**.

Table 1: List of LipL21 sequences of *Leptospira* species selected from BLAST search

S. No.	Organism	Accession No.	Identity percentage	Max. score	Query coverage	e-value
1	*Leptospira interrogans serovar Autumnalis*	AMT00570.1	100.00%	365	100%	4e-130
2	*Leptospira interrogans serovar Ballum*	AJF45998.1	100.00%	365	100%	5e-78
3	*Leptospira interrogans serovar Pomona*	ABA10364.1	100.00%	364	99.44%	1e-129
4	*Leptospira interrogansserovar Mini*	ABA10366.1	100.00%	363	99.44%	2e-129
5	*Leptospira interrogansserovar Manhao II*	ABA10365.1	100.00%	362	99.44%	6e-129
6	*Leptospira interrogans*	WP_000610517.1	100.00%	363	98.88%	3e-129
7	*Leptospira noguchii*	WP_002179282.1	100.00%	362	98.32%	7e-129
8	*Leptospira gomenensis*	WP_135593739.1	100.00%	362	98.32%	9e-129
9	*Leptospira elllisii*	WP_100746353.1	100.00%	361	97.77%	2e-128
10	*Leptospira krischneri*	WP_025178454.1	100.00%	360	98.32%	3e-128
11	*Leptospira mayottensis*	WP_036047830.1	100.00%	360	98.32%	6e-128
12	*Leptospira alexanderi*	WP_010577422.1	100.00%	359	97.77%	1e-127
13	*Leptospira weilii*	WP_036096255.1	100.00%	358	97.21%	2e-127
14	*Leptospira barantonii*	WP_100763498.1	100.00%	358	97.77%	2e-127
15	*Leptospira borgpetersenii*	WP_011669143.1	100.00%	358	97.77%	3e-127
16	*Leptospira kmetyi serovar Malaysia*	AJF45994.1	100.00%	356	97.21%	1e-126
17	*Leptospira kmetyi*	WP_010573134.1	100.00%	356	97.21%	1e-126
18	*Leptospira santarosai*	WP_061215473.1	100.00%	356	96.65%	1e-126
19	*Leptospira alderi*	WP_100784522.1	100.00%	355	97.21%	3e-126
20	*Leptospira tipperaryensis*	WP_069605664.1	100.00%	353	96.09%	3e-125
21	*Leptospira alstonii*	WP_036040502.1	100.00%	347	94.41%	5e-123
22	*Leptospira semungkisensis*	WP_135586463.1	100.00%	318	86.03%	2e-111
23	*Leptospira lagatesnsis*	WP_135645437.1	100.00%	316	85.41%	8e-111
24	*Leptospira broomii*	WP_010571520.1	100.00%	316	84.92%	8e-111
25	*Leptospira perolatii*	WP_100712538.1	100.00%	313	83.80%	2e-109
26	*Leptospira wolffii*	WP_100758258.1	100.00%	311	84.36%	7e-109
27	*Leptospira dzoumogneensis*	WP_135758075.1	100.00%	309	82.68%	6e-108
28	*Leptospira haakeii*	WP_100722355.1	100.00%	231	54.34%	3e-77
29	*Leptospira saintgironsiae*	WP_100709232.1	100.00%	207	52.75%	2e-67
30	*Leptospira kemammanesis*	WP_135587940.1	100.00%	159	46.88%	1e-48
31	*Leptospira biflexa*	WP_012387047.1	100.00%	159	46.88%	1e-48
32	*Leptospira monotravelensis*	WP_135572710.1	100.00%	159	46.88%	2e-48
33	*Leptospira bandrabouensis*	WP_015677091.1	100.00%	157	46.88%	5e-48
34	*Leptospira idonii*	WP_135759194.1	100.00%	153	46.35%	3e-46

Table 2: Physico-chemical properties of the LipL21 protein sequences

Properties	LipL21
No. of Amino acids	179
Molecular wieght	18890.19
pI	7.54
Extinction coefficients	28210
Instability index	32.68
Aliphatic index	59.94
Gravy	-0.425

Table 3: Prediction of hydropathicity of LIPL21 sequences

Accession no.	Protein name	Average Hydrophobicity	Protein type
A0A144I2F4	**LipL21**	**-0.425140**	**Soluble protein**

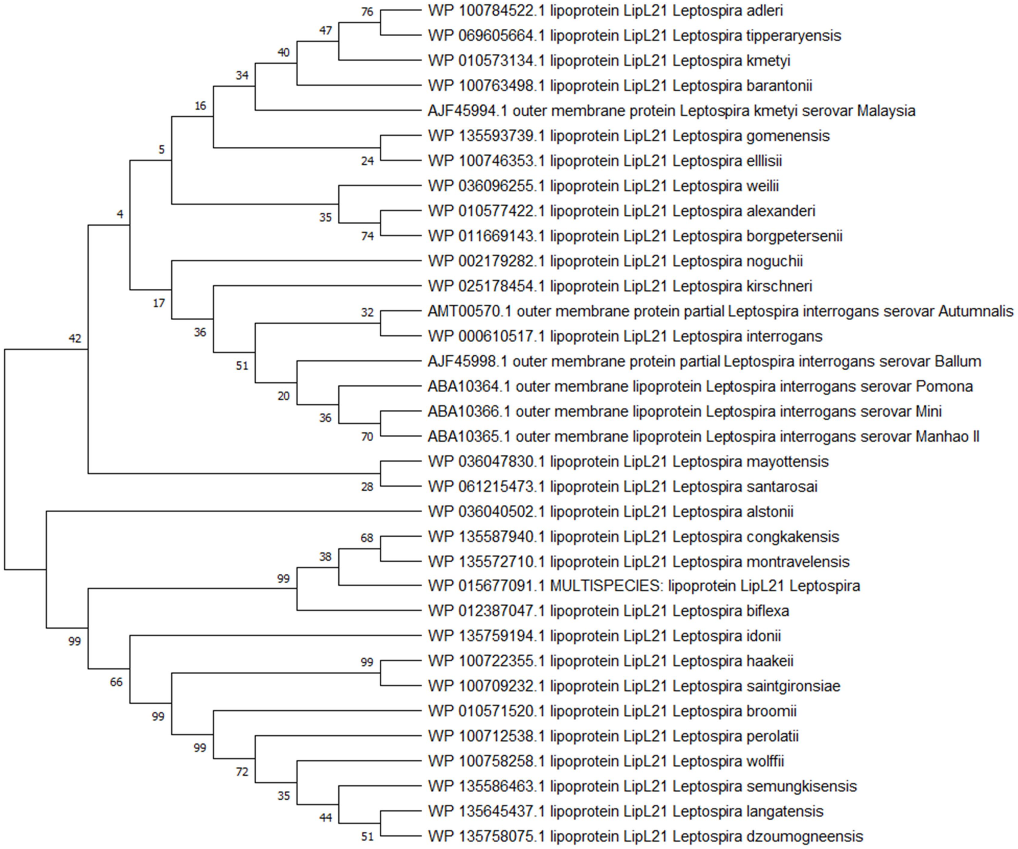


Figure 1: Phylogenetic tree with 34 sequences of LipL21 within different species of *Leptospira*

Table 4: Secondary structural features of LipL21 protein using GOR IV

Protein	Random Coil (%)	Extended Strand (%)	Alpha Helix (%)
LipL21	**49.16**	**36.87**	**13.97**

Table 5: Tertiary structure prediction and 3-model validation

Organism	Accesion No.	Template ID	Identity	Query coverage	Rampage		
					Number of residues in favored region	Number of residues in allowed region	Number of residues in outlier region
***Leptospira interrogans* serovar Autumnalis**	**LipL21**	**5ok8.3.A**	**14.16%**	**32-129**	**91.7%**	**6.2%**	**2.1%**

Figure 2: 3D-model of LipL21 from *Leptospira interrogans* serovar autumnal using SWISS-MODEL workspace

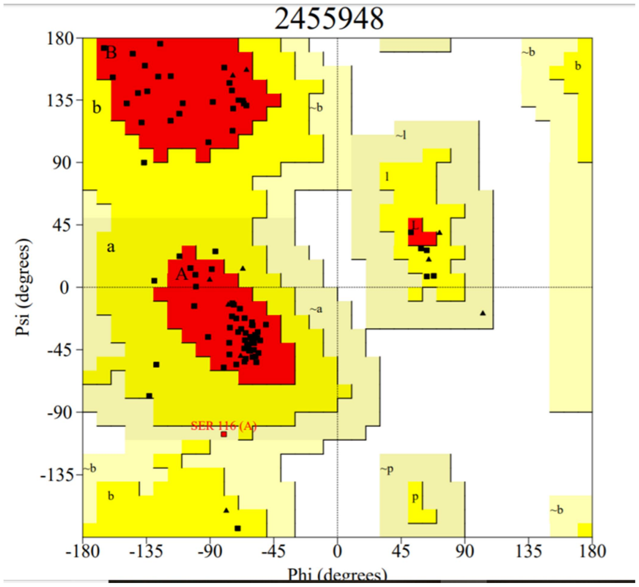
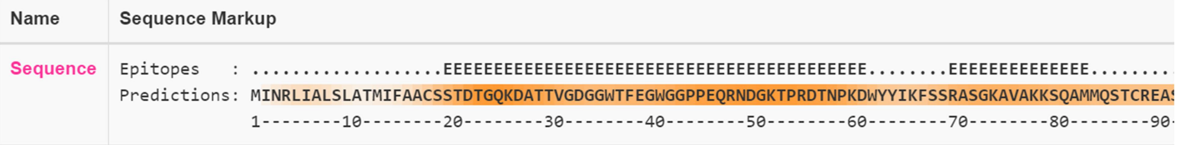
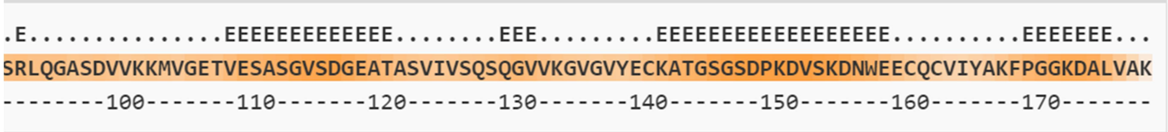


Figure 3: Ramachandran plot of LipL21 model. The most favoured region arecolored red, addition allowed, generously allowed and disallowed regions are indicated as yellow, light yellow and white respectively



```
Name       Sequence Markup
Sequence   Epitopes   : ...................EEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEE.......EEEEEEEEEEEEEEE.......
           Predictions: MINRLIALSLATMIFAACSSTDTGQKDATTVGDGGWTFEGWGGPPEQRNDGKTPRDTNPKDWYYIKFSSRASGKAVAKKSQAMMQSTCREA
                        1--------10--------20--------30--------40--------50--------60--------70--------80--------90
```

(a)

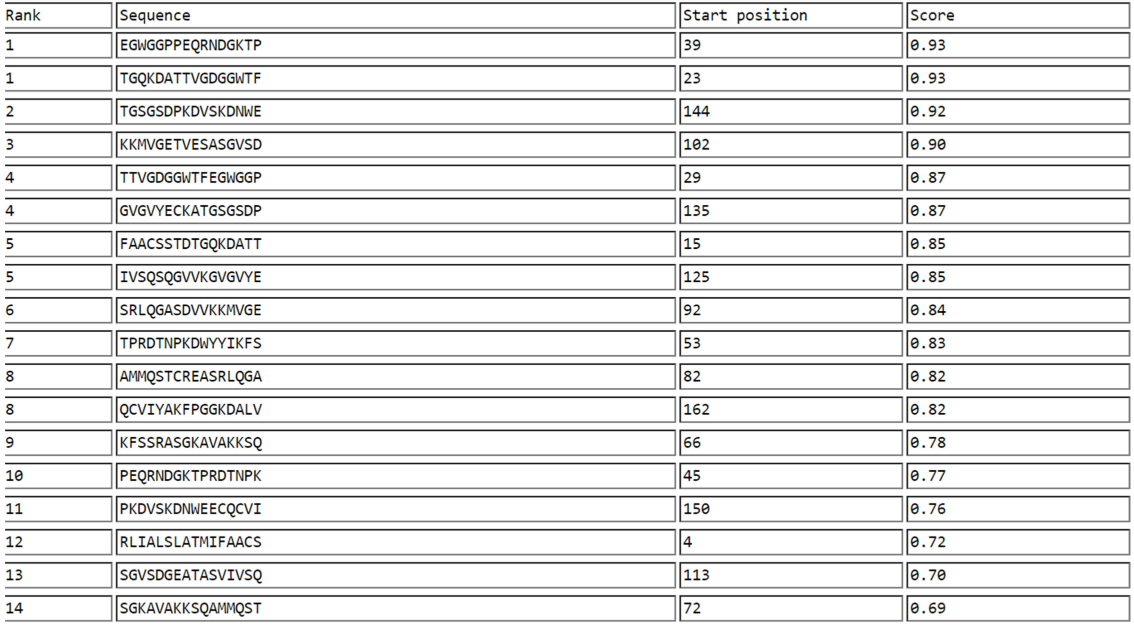


```
.E...............EEEEEEEEEEEEE........EEE.........EEEEEEEEEEEEEEEEEE..........EEEEEEE...
SRLQGASDVVKKMVGETVESASGVSDGEATASVIVSQSQGVVKGVGVYECKATGSGSDPKDVSKDNWEECQCVIYAKFPGGKDALVAK
--------100-------110-------120-------130-------140-------150-------160-------170-------
```

(b)

Figure 4: (a) Potential B-cell epitope from range from 1-90 amino acid by BepiPred;
(b) Potenital B-cell epitope range from 91-177 amino acid by BepiPred

Table 6: Epitope prediction by using ABCpred



Rank	Sequence	Start position	Score
1	EGWGGPPEQRNDGKTP	39	0.93
1	TGQKDATTVGDGGWTF	23	0.93
2	TGSGSDPKDVSKDNWE	144	0.92
3	KKMVGETVESASGVSD	102	0.90
4	TTVGDGGWTFEGWGGP	29	0.87
4	GVGVYECKATGSGSDP	135	0.87
5	FAACSSTDTGQKDATT	15	0.85
5	IVSQSQGVVKGVGVYE	125	0.85
6	SRLQGASDVVKKMVGE	92	0.84
7	TPRDTNPKDWYYIKFS	53	0.83
8	AMMQSTCREASRLQGA	82	0.82
8	QCVIYAKFPGGKDALV	162	0.82
9	KFSSRASGKAVAKKSQ	66	0.78
10	PEQRNDGKTPRDTNPK	45	0.77
11	PKDVSKDNWEECQCVI	150	0.76
12	RLIALSLATMIFAACS	4	0.72
13	SGVSDGEATASVIVSQ	113	0.70
14	SGKAVAKKSQAMMQST	72	0.69

Table 7: List of LipL21 epitope predicted based on amino acid sequence

Protein	Start Position	End position	Peptide	Number of residues	Score
LipL21	**69**	**81**	**SRASGKAVAKKSQ**	**13**	**0.777 (Probable Non-antigen)**
	107	**125**	**ETVESASGVSDGEATASVI**	**19**	**0.711 (Probable Non-antigen)**
	48	**62**	**RNDGKTPRDTNPKDW**	**15**	**0.657 (Probable Non-antigen)**

The predicted peptides from various immunoinformatic servers is significant not only for diagnostics but also for vaccines. It became clear that the small segments of protein called the antigenic determinants, or the epitopes are sufficient for eliciting the desired immune response. All these epitopes predicted can be used for the development of monoclonal antibody or epitope based diagnostic kit for Leptospirosis after further experimental and validation. The prediction and immunoinformatic analysis of antigenic peptideswere done by using three different programs for epitope prediction. These software's were used to predict immunogenic linear B-cell epitopes on the surface of *Leptospira*l outer membrane protein LipL21.

CONCLUSION

In our investigation, the structural and functional characterization of the protein LipL21 from *Leptospira interrogans* serovar autumnal was performed to find out their functional properties, physicochemical properties and structural modelling at different level. Primary structural analyses revealed that the proteins were hydrophilic and were expected to be stable over a wide range of temperature. Secondary structure analysis established that in most of the sequences, random coils were dominating, followed by alpha helix, extended strand and beta turns. Protein 3D modeling was predicted to provide insight into the structural properties of polypeptide chains with the significance for understanding of protein folding and molecular design. The prediction of protein structural features played a vital role in virulence process and exposed on the surface of the *Leptospira* helped in the identification of candidate antigens in protective immune response. Three B-cell epitopes were predicted from LipL21 which can be used as therapeutic agents to combat Infection. Antibody developed against these conserved epitopic regions could be used to develop a detection kit or as a vaccine candidate for Leptospirosis after experimental validation.

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