Life Science Informatics Publications

Research Journal of Life Sciences, Bioinformatics, Pharmaceutical and Chemical Sciences

Journal Home page http://www.rjlbpcs.com/

RJLBPCS

ISSN 2454-6348

Original Research Article **DOI: 10.26479/2019.0504.07**

IN-SILICO ANALYSIS OF THE *UREAPLASMA UREALYTICUM* PROTEOME CAUSING INFERTILITY

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ABSTRACT: *Ureaplasma urealyticum* is a gram-negative bacterium usually found in the urogenital tracts of human and causes infections like vaginosis. The bacterium is also associated with number of infectious diseases which affects neonates and pregnant women. The untreated bacterial infections can lead to the sequalae like pelvic inflammatory disease (PID), recurrent pregnancy losses, ectopic pregnancy and infertility. The present study focuses on analysis of the proteins available for the pathogen. All the protein sequences of pathogen were collected and examined using various in silico methods to identify the most immunogenic proteins that are membrane bound, non-allergens and have functional domains.

KEYWORDS: *Ureaplasma urealyticum,* infertility, swiss model.

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1.INTRODUCTION

Ureaplasma urealyticum is a gram-negative bacterium frequently found in urogenital tracts of humans [12] that is usually associated with the Mycoplasmas. The unique feature of it is the ability to generate ATP by hydrolysis of urea [22]. The bacterium is often a part of normal flora in the reproductive tract of sexually active men and women [21]. Many a times women with this infection experience fertility problems, the untreated infections can render to infertility [15]. Ureaplasma species can cause acute urethritis and have been associated with bacterial vaginosis, preterm birth, and neonatal respiratory disease [4]. Number of reports discuss the association of *U.ureaplasma* with prematurity-linked conditions like preterm labour, premature rupture of the fetal membranes, placental invasion and intra amniotic infection as well as chorioamnionitis, postpartum and postabortal fever [25]. The infected mothers can transmit the pathogen to their babies and the new-

borns which get infected are prone to diseases like meningitis, pneumonia and respiratory tract disorders [27]. Cervical discharge due to infection in upper genital tract by the pathogen which results in secondary infertility [6]. *Ureaplasma* are self-replicating organisms that colonises in human and has the capacity to utilize urea for generation of ATP [3]. There are a number of pathogens known to contribute to male infertility; the two types that most commonly occur are genital *ureaplasma* and *mycoplasma* [13]. They are ubiquitous resulting in colonisation of the genitalia by sexual contact [2]. Several studies have demonstrated that *U. urealyticum* and *M. hominis* play an etiologic role in male infertility, with these infections changing parameters of semen such as spermatozoa density and motility [17]. However, a significant relationship existed *between U. urealyticum and M. hominis* and male infertility [11].

2. MATERIALS AND METHODS

2.1 Protein sequence retrieval and identification of antigenic protein

All the 646 protein sequences of *Ureaplasma urealyticum* were retrieved in FASTA format from UniProt Proteome database www.uniprot.org [1]. Antigenic proteins of *U.urealyticum* were identified using VaxiJen server v2.0 [8].

2.2 Evaluation of allergenic, sub-cellular localization and functional analysis of proteins.

The antigenic proteins were further evaluated for their allergenicity using AlgPred [23]. The allergens were excluded for further analysis. The sub-cellular localization predictions of *U.urealyticum* protein sequences was done using SOSUIGramN tool [14] as the pathogen lacks cell wall. Then the structural and functional analysis was performed for the sequences by Protparam [10] and InterPro [9] for obtaining the physical and chemical parameters of the proteins and identification of the conserved domains and important sites in the proteins.

2.3 2D, 3D Protein structure modelling and Validation of the models.

Due to the lack of structures in the database homology modelling was performed for the proteins. The 2D structure prediction for the sequences was performed using Self-Optimized Prediction Method with Alignment SOPMA [5]. And the 3D structures were obtained by protein modelling using SwissModel [28]. The quality of the homology model was then validated using online tools such as Rampage [20] and Procheck [16].

3. RESULTS AND DISCUSSION

A total of 646 protein sequences of *U. ureaplasma* were collected from the UniProt Proteome database the sequences were set to series of screening analysis.

3.1 Antigenic protein prediction

Out of all proteins of the pathogen, 435 protein sequences in the study were screened out based on the antigenicity score. The threshold of 0.4 was considered as the potent antigenicity score. All the proteins with higher prediction scores more than 0.500 were considered as antigenic and were selected for further analysis.

3.2 Evaluation of allergenic, sub-cellular localization and functional analysis of proteins.

Algpred allows prediction of allergens based on similarity and mapping of IgE epitopes with any region of protein. The non-allergenic proteins were considered for further analysis. The SOSUI-GramN tool was used for screening the protein sequences, the tool predicted outer and inner membrane proteins and extracellular proteins. The proteins which are antigenic, non-allergenic and membrane bound were taken for further analysis. The physical and chemical parameters for the given proteins were computed using ProtParam Expasy tool. The computed parameters for the proteins include the molecular weight, theoretical pI, number of amino acids, amino acid composition, atomic composition, extinction coefficient, estimated half-life, aliphatic index, instability index, and grand average of hydropathicity (GRAVY).

3.3 2D and 3D Protein structure modelling and Validation of the models.

The secondary structural elements of the proteins were predicted using SOPMA. In the figure the blue line indecates the helices, red indicates beta sheets, green indicates the beta turns, and purple indicates ambiguous and other states.

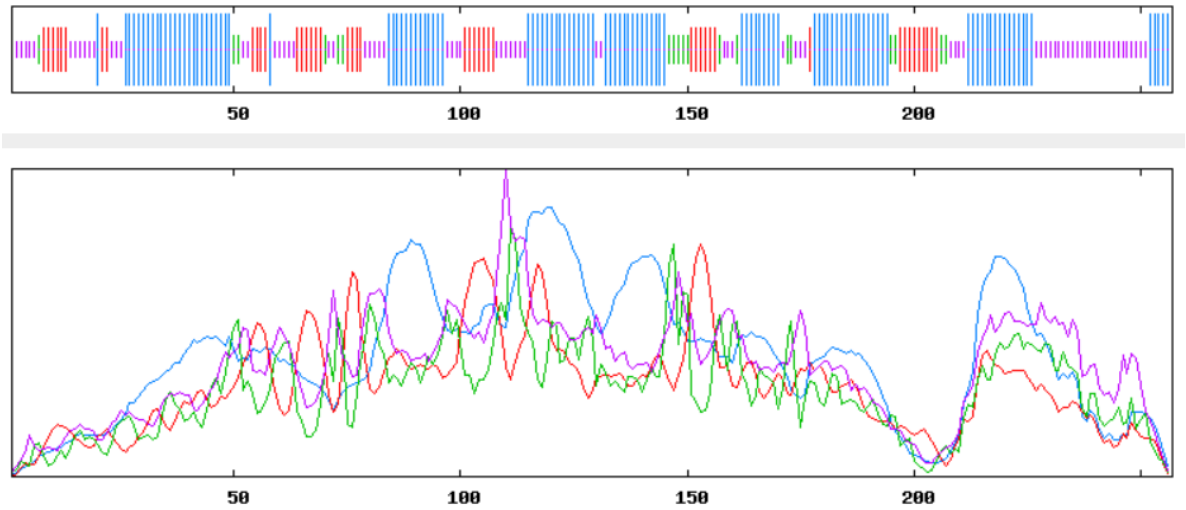

Figure 1: SOPMA results for the protein the blue line indecates the helices, red indicates beta sheets, green indicates the beta turns, and purple indicates ambiguous and other states.

Followed by 2D structure prediction 3D structure prediction wasdone using Swiss Model with QMEAN -3.52. The structures were validated using RAMPAGE and PROCHECK which produces the PostScript Ramachandran plots for analysing overall residue by residue geometry given in the figure 2 shows the results of Swiss model with structure and its QMEAN analysis. Figure 3 shows the Procheck Ramachandran plot validation for the protein.

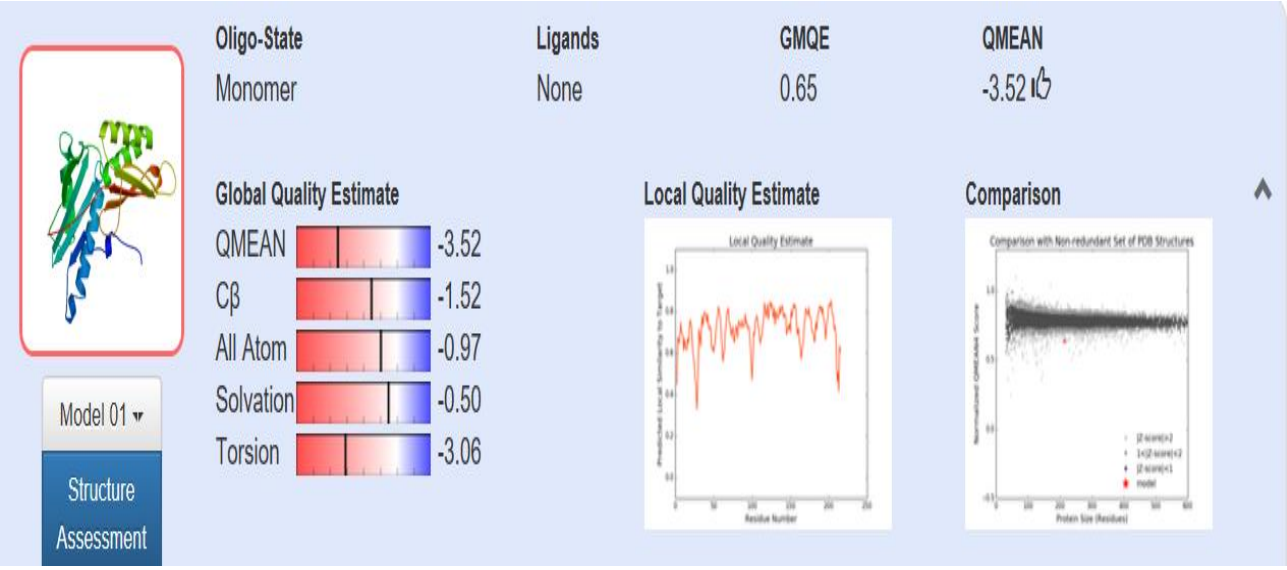

Figure 2: SwissModel results for the protein 30S ribosomal protein with QMEAN -3.52.

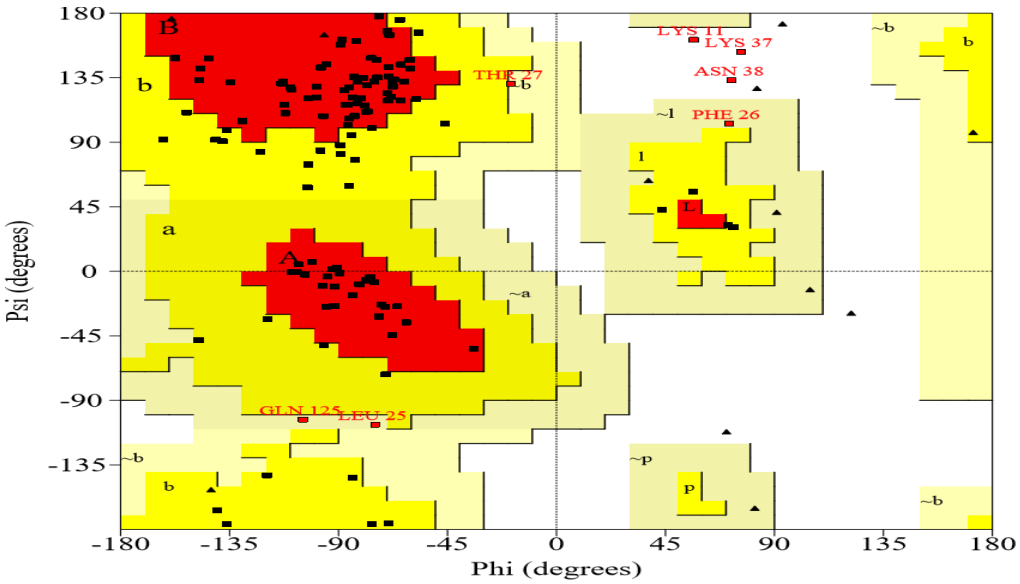

Figure 3: Ramachandran plot analysis of the protein using Procheck.

DISCUSSION

The current study identifies the potential proteins that are highly antigenic, membrane bound, non-allergenic that have conserved domains which perform vital functions in the organism. 50S Ribosomal protein L2 is evolutionarily highly conserved protein, which is involved in catalysis of peptide bond formation during transcription. Also, it brings about association between 50S and 30S subunits and helps in binding of tRNA to Active and Peptide formation sites in the ribosomes [7]. The oxidase assembly (OXA) translocase is required for exporting mitochondrial-encoded proteins; however, we report that OXA plays a dual role in the biogenesis of nuclear encoded mitochondrial proteins. First, a systematic analysis of OXA-deficient mitochondria led to an unexpected expansion of the spectrum of OXA substrates imported via the PR sequence pathway. Second, biogenesis of

numerous metabolite carriers depends on OXA, although they are not imported by the pre-sequence pathway [24]. The study involved retrieval of the whole proteome of the pathogen followed by screening for antigenic proteins as antigenicity determines the virulence of the pathogen [19]. The non-antigenic proteins were excluded for further analysis, antigenic proteins were further analysed for their localization and allergenicity. The membrane bound proteins are easily accessible to the drugs [26]. Non allergens were selected so that they do not generate the side effects or allergic reactions [18]. The proteins 50s ribosomal protein, Putative membrane protein, Membrane protein OxaA, Phosphate transport protein are membrane bound with 0.5513, 0.7570, 0.7323, 0.8555 as antigenicity scores. Further the protein functional analysis using ProtParam and InterPro was done for the proteins 50s ribosomal protein has 279 amino acids with molecular weight of 30872.55, theoretical pI 10.40, extinction coefficient 15930, half life estimated 30 hrs, instability index 46.85, aliphatic index 80.68 and GRAVY -0.675. InterPro identified the domains in the proteins, all the proteins have conserved domains ranging from 3 to 5. Secondary structure prediction was done using SOPMA, the proteins. The protein has more no. of helical and beta sheet residues. The tertiary structure prediction was done using SwissModel for proteins. The protein models were validated for the structural conformations using the validation tools Procheck and Rampage. The proteins 50s ribosomal protein, Putative membrane protein, Membrane protein OxaA, Phosphate transport protein showed 90.02, 92.3, 90.6, 93.3 as their validation scores indicating more then 90 percent of the amino acids are in the allowed regions of the Ramachandran Plot. The Procheck result for 50s ribosomal protein is shown above indicating a greater number of amino acids in favoured regions.

The study identifies the proteins that are antigenic, membrane bound, non-allergenic which can be opted during the drug designing process as druggable targets for the pathogen. The functional analysis and secondary structure analysis also indicate good results for selecting them as targets. Further molecular docking analysis of the specified targets with ligands can be performed to validate the identified targets.

4. CONCLUSION

Adequate treatment options for the infectious pathogens helps in timely cure of the patients. The pathogen *Ureaplasma urealyticum* causes sexually transmitted infections. In the current study the effort is made to identify the targets for the pathogen *Ureaplasma urealyticum.* The proteins 50s ribosomal protein, Putative membrane protein, Membrane protein OxaA, Phosphate transport protein were identified that are more antigenic and membrane bound that are suggested targets in drug designing process.

ACKNOWLEDGEMENT

Thanks to DBT BIF centre, department of Bioinformatics, Karnataka State Akkamahadevi Women's University, Vijayapura for support and encouragement.

CONFLICT OF INTEREST

The authors Shilpa Shiragannavar and Shivakumar Madagi declare that they have no conflict of interest.

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