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IN SILICO STUDIES ON rpsL GENE PRODUCT RESPONSIBLE FOR VECTOR-BORNE TROPICAL AND SUBTROPICAL DISEASE FILARIAL ELEPHANTIASIS

MARYADA ROY^{*1,2}, PALLAVI GANGWAR², PRASHANTA KUMAR PAL¹ AND ANKUR MOHAN²

1: Mangalayatan University, Aligarh-Mathura Road, Aligarh, Uttar Pradesh, India

2: Department of Life Sciences, Bio-EGICORE, Lucknow, Uttar Pradesh, India

ABSTRACT

Filarial elephantiasis (lymphatic filariasis), a vector-borne tropical disease has known to be endemic in various regions of India as well as Africa, Philippines, Fiji, Latin America, China, Japan, Thailand, Korea, Sri Lanka. It is mainly caused by helminthes (nematodes) parasitic worms namely *Wuchereria bancrofti*, *Brugia malayi* and *Brugia timori*, which are able to invade the lymphatic system of the human host via suitable mosquito vector namely *Culex quinquefasciatus*, *Anopheles species*, *Aedes species*, *Mansonia species*. The larvae of the worm transmitted by insect inhabit the lymph nodes (mainly inguinal nodes), causing fever, inflammation of the lymph vessels, and years latter, thickening (elephantiasis) of the lower extremities, scrotum, mammary glands take place. An endosymbiotic gram negative bacterium, *Wolbachia pipientis*, plays an important role, by establishing a symbiotic relationship with these worms, in their growth and development inside the host body. Present study is based on an *in silico* approach in which the gene, rpsL has been targeted. The gene rpsL encodes 30s ribosomal proteins, in *W. pipientis*, to contribute in the prevention and cure of filarial elephantiasis. With the help of suitable bioinformatics tools, a suitable 3-dimensional protein model of the query protein has been designed. Sequence alignment, structure prediction and validation of predicted model (loop modeling) of the protein with the available protein sequences have been submitted to the computational database. The final selected template protein has been used to prepare a most probable computational model of the targeted protein molecule(s).

Keywords: Lymphatic Filariasis, helminthes, *Culex quinquefasciatus*, *Wolbachia pipientis* Bioinformatics, in silico studies, Ramachandran Plot, BLASTp, 3DPSSM, Phyre-2, Easy MODELLER, PROCHECK, Swiss PDB Viewer

INTRODUCTION

Human filarial elephantiasis, commonly known as lymphatic filariasis, is a mosquito-mediated parasitic disease which is caused by thread-like filarial worms, mainly *Wuchereria bancrofti*, *Brugia malayi*, *Brugia timori* [2, 3, 24]. These nematode worms belong to superfamily filarioidea and family Onchocercidae; inhabit the lymphatic and subcutaneous tissues of the host organism, develops physical and social disability [11, 19]. The adult worms (macrofilariae) inhabit human lymphatic system (lymph nodes and lymphatic vessels), where they generally survive for about 5-10 years and in some case, for about 15 years. The female worms reproduce millions of immature microfilariae (mf) after mating, during their lifespan, which finally migrate towards the blood stream of the host organism. These sheathed mfs require about six months to one year after infection (pre-patent period) to appear in the blood stream (maturation period). During the day, they shelter into the arterioles and the peripheral circulation at night (nocturnally periodic). Mf possess cyclo-developmental life cycle inside the vector organism. After engorging by mosquito vector, by taking blood meal of an infected person, mf requires 10-14 days to develop into infective larvae, inside the vector. They do not multiply during their

life cycle. Besides it, they penetrate the stomach wall after shedding their protective sheathes, migrate towards thorax for their development. The expected mean length of the slender and tiny mf of *W. bancrofti*, *B. malayi* and *B. timori* is 290 μ , 222 μ , 310 μ . They are capable in transforming to an immobile and sausage stage (L1) which is an inactive form having a conspicuous slender tail, developed by cuticle, with specific identification characters. The L1 stage mf again undergoes into the next step of development stage by making growth in length and breadth, to form a pre-infective larvae (L2) stage. The L2 develops into harboring L3 stage, which is infective, slender, highly motile, thread-like, having measurement of about 1500-2000 microns in length. This infective larvae, then develops into adult male and female worms inside the lymphatic system of the host [3, 6].

Lymphatic filariasis spreads with a mosquito vector, an intermediate host [6]. *Culex quinquefasciatus*, *Anopheles species*, *Aedes species*, *Mansonia species*. Brugian filariasis is transmitted by *Mansonia bonnea* and Bancroftian filariasis is transmitted by either *Aedes poicilus* or *Anopheles minimus flavirostiris* in Phillipines; besides it, studies have found that *Cx. quinquefasciatus* is a poor vector of bancroftian filariasis in

Phillipines [5]. *Cx. quinquefasciatus* is found in India and widespread in the world [3]. *Anopheles gambiae* and *Anopheles funestus* has been found as an important vector in West Africa, *Aedes aegypti* and *Aedes pambaensis* in East Africa; *Mansonia uniformis* and *Mansonia africanus* in Egypt [11]. In India, Brugian filariasis (*B. malayi*) spread by Mansonian spp., namely *M. Indiana*, *M. uniformis*, *M. annulifera* and Bancroftian filariasis is transmitted by *Cx. quinquefasciatus*; in Nicobar island, *Aedes (finlaya) niveus* has found to be the subperiodic vector of *W. bancrofti* [1].

Lymphatic filariasis is an endemic tropical disease which extends to subtropical regions as well [11, 20, 22]. Its clinical manifestations vary according to the geographical regions [4]. The most common clinical symptoms found in the patients include fever, acute adenolymphangitis, lymphedema (of arms, legs, breasts, male and female genitals), chyluria, hydrocele, tropical pulmonary eosinophilia [1, 2, 19, 8, 4]. The larvae of the worm transmitted by insect inhabit mainly the inguinal nodes, causing fever, inflammation of the lymph vessels, and years later, thickening (elephantiasis) of the lower extremities, scrotum, mammary glands take place [29]. The medicines for prevention of filariasis should be used only under supervision of a clinician, since the drugs for treatment and

prophylaxis of the disease are likely to cause side effects [29].

After filarial infection, the host body generates an immune response in which cell mediated immune response and high level of inflammatory cytokines have been observed [28]. *Wolbachia pipientis*, an endosymbiotic bacterium has been found to play a major role in generating immune response in the host body [28]. Endosymbiotic *Wolbachia* is a gram negative bacterium which has been found in Bancroftian filarial infection, helps in the development of infective microfilariae by establishing a symbiotic relationship with the filarial worms [26, 27].

In India, filarial elephantiasis is majorly endemic in the regions of West Bengal, Orissa, Andhra Pradesh, Bihar, Kerala, Tamil Nadu, Gujarat, Uttar Pradesh [4, 12, 13, 15] with minor presence in the regions of Andaman and Nicobar, Maharashtra, Goa, Assam, Lakshadweep [1, 4]. Filarial infection has covered various districts of west coast of Kerala including Trichur, Ernakulum, Alleppey, Kottayam, Quilon and Trivandrum, spreading over an area of approximately 1800 square kilometer [4]. Infection of *W. bancrofti* has been found in the five districts of Orissa, namely Bargarh, Sambalpur, Balangir, Kandhamal, Kendujhar [15] as well as Central India i.e. Raipur city of Chhatisgarh [23]. In West

Bengal, filarial infection has been reported in Parganas district [12], Purba Medinipur district [13], West Midnapur district [17], Murshidabad district [25], Bankura and Birbhum district [27]. Saidapet has found to be heavily infected area near Madras, Tamil Nadu [10].

Lymphatic filariasis is a global disease with presence in other countries across the world. It has been reported from Cross River State [8], Imo State of Nigeria [2], in the region of Phillipines islands, majorly found in three islands, namely central Luzon, Visayas and Mindanao [5], Fiji [10], Eastern and Southern African countries (according to the report of WHO) which include Uganda, Ethiopia, Kenya, Tanzania, Seychelles, Mauritius, Comoros, Madagascar, Mozambique, Eritrea, Malawi, Zambia, Zimbabwe, Namibia, Botswana, Lesotho, south Africa, Swaziland [11], sub-Saharan Africa, Asia, tropical islands of Pacific Ocean, focal areas of Latin America, Papua New Guinea [18], China, Japan, Thailand, Korea, Solomon Island, Sri Lanka (especially infected with Brugian filariasis), Costa Rica, Suriname, Malaysia, Brazil, Trinidad and Tobago [21], and also near Caribbean Sea [22]. Bancroftian filariasis has been found in 37 districts of Nepal including Bardiya, Surkhet, Banke, Dang, Palpa, Kaski, Tanahu, Gorkha, Chitwan, Sindhuli, Jhapa, Kavre, Palpa [20]. It has

been found that hydrocele is not seen with Brugian infection while lymphoedema occurs in all filarial infections. Rare visibility of pulmonary eosinophilia and chyluria has been observed [4]. In China, the clinical symptoms of Chyluria (intermittent leakage of intestinal lymph into the renal pelvis and consequently in the urine) has been observed than lymphoedema while in Africa, cases of lymphoedema has been more observed [7]. In spite of such a global presence, lymphatic filariasis has been mostly studied in a traditional way. A genomic, proteomic and rational approach towards drug discovery is much sought for.

Present research work is a novel approach, with the help of bioinformatics tools, the 30s ribosomal protein in *Wolbachia pipientis*, encoded by rpsL gene, has been chosen. A 3-dimensional structure has been modeled. This model along with the sequence homology and loop modeling can be used to develop a *de novo in silico* drug targeted against 30s ribosomal protein responsible for lymphatic filariasis.

METHODOLOGY

Sequence Alignment

The FASTA sequence of 30s ribosomal query protein, encoded by rpsL gene has been obtained from online source of National Centre for Biotechnology Information (<http://www.ncbi.nlm.nih.gov>) and aligned by using BLASTp sequence

alignment tool (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>).

Chain O, Crystal structure of 70s ribosome (PDB ID: 4G5K), contain the 30s ribosomal subunit, has showed maximum identity with the query protein. Hence, it has been selected as a template protein for structure prediction of the targeted protein molecule [9].

Structure Prediction

The three dimensional secondary structure of 30s ribosomal protein has been predicted by 3DPSSM and Phyre 2, an online server. With the help of these servers, a few protein templates have been selected (PDB ID: 1RUT, 1W98, 2IX0, 2UUB, 2VO1) which were showing maximum identity with our query protein structure. The PDB file format of these template proteins, possess resolution <3.0, R-Value <5.0 and X-Ray crystallographic structure, have been obtained from Protein Data Bank, a computational database. The 3-dimensional structure of query protein has been obtained by submitting PDB file formats of these template protein in a unique, automated bioinformatics software, EASY MODELLER (version 3.0), a user guided computational tool used for comparative and homology modeling of three dimensional, flexible structure of protein containing all non hydrogen atoms [9].

Validation of Predicted Model (Loop Modeling)

Validation of the predicted 3-dimensional model of desired protein has been performed with Ramachandran plot analysis by further submitting the model to rebuild it into its secondary structure, with the help of an online server, SAVES server (PROCHECK). The final secondary structure has been verified and viewed in Swiss PDB Viewer (SPDB 4.10) [9].

RESULTS AND DISCUSSION

Homology modeling or comparative modeling of protein molecule provides a flexible three dimensional structure of targeted protein molecule which provides a platform for designing the best drug molecule targeted against the modeled protein and for the study of *protein-drug interaction in silico* [14, 16]. In this research work, the three dimensional model of 30s ribosomal protein encoded by rpsL gene of bacterium *W. pipientis*, has been designed by using EASY MODELLER 3.0. The initial step for protein modeling is to obtain the protein sequence from NCBI and to perform alignment of the query sequence with the available protein sequences by BLASTp. In the result we found 74% identity with the Chain O of Crystal structure of 70s ribosome with tetracycline, that contains 30s ribosomal subunit (**Figure 1, 2**). This sequence was, then, taken as a

template sequence from which a three dimensional structure has been obtained and that structure has been aligned by using online server 3DPSSM and PHYRE 2 (**Figure 3**). The final selected template protein has been calculated for the dope score by EASY MODELLER 3.0 (**Figure 4**). The template having dope score - 5853.56104 has been selected for loop modeling (**Figure 5**) being very close to satisfy the properties of Ramachandran plot used for analysis of protein structure, obtained from an online server, SAVES (PROCHECK). The loop modeling was successfully done by using SPDBV 4.10 (**Figure 6**) and the final loop modeled structure was obtained which completely satisfied the determining properties of a protein structure described in Ramachandran plot (**Figure 7, 8**).

CONCLUSION

In the present study, an *in silico* protein model of 30s ribosomal protein, which is a product of rpsL gene synthesized within a bacterium, *Wolbachia pipientis* has been designed by using suitable bioinformatics software and online servers. Studies have clearly showed that *W. pipientitis* plays a major role in the development of microfilariae into macrofilariae of filarial worms inside the body of host. Therefore, this study can be beneficial for novel computational drug designing by taking the

above modeled protein as a target, to develop treatment or therapy of lymphatic filariasis.

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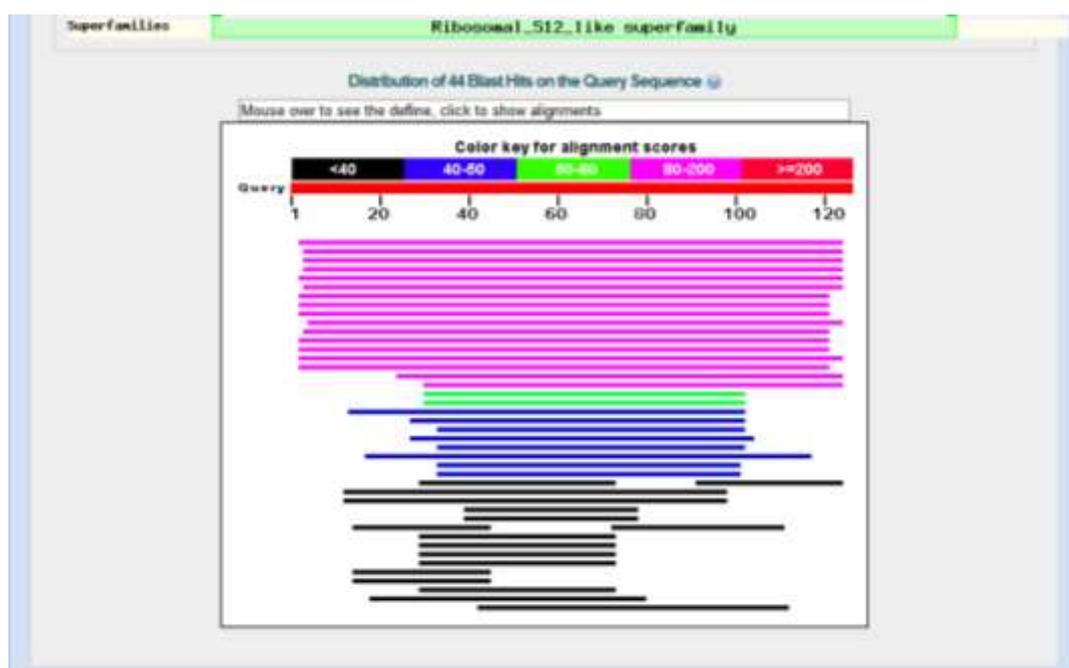


Figure 1: Blast Results for alignment of Query Protein with the protein Sequences available on NCBI

Sequences producing significant alignments:

Select: All None Selected: 0

Alignments

Description	Max score	Total score	Query cover	E value	Ident	Accession
Chain O, Crystal Structure Of The 70s Ribosome With Tetracycline. This Entry Contains The 30s Sub	174	174	96%	6e-56	74%	4QSK_O
Chain L, Thermophilus 70s Ribosome In Complex With Mna, Trna And Ef-Tu Gdp Kimerico Tr	171	171	96%	6e-55	74%	3FIC_L
Chain L, Crystal Structure Of The Thermus Thermophilus 30s Ribosomal Subunit Complexed With /	171	171	96%	6e-55	74%	4K0K_L
Chain O, Coordinates Of S12, L11 Proteins And P-Trna, From The 70s X-Ray Structure Aligned To T	171	171	96%	7e-55	74%	1FNZ_O
Chain L, Crystal Structure Of The Bacterial Ribosome From Escherichia Coli In Complex With The A	171	171	96%	7e-55	70%	1V35_L
Chain L, Crystal Structure Of The 70s Ribosome Bound With The Q253e Mutant Of Release Factor E	171	171	96%	8e-55	74%	4K2H_L
Chain L, 30s Ribosome + Designer Antibiotic -pdb12HG10 Chain O, Crystal Structure Of The 70s Th	171	171	94%	1e-54	74%	2E4V_L
Chain L, Structure Of The Thermus Thermophilus 30s Ribosomal Subunit In Complex With The Antl	169	169	94%	3e-54	73%	1F3Q_L

Figure 2: Showing Percentage Identity between Query Protein and Available Protein Sequences After Running BLASTp

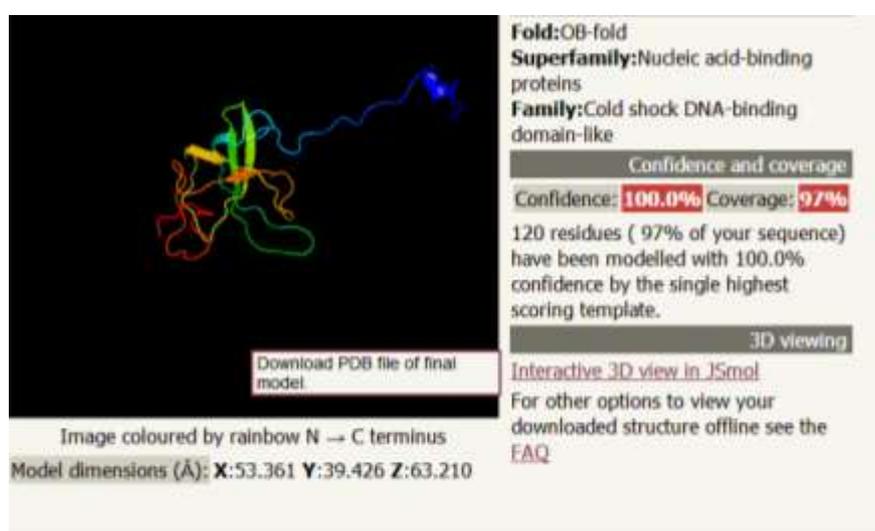


Figure 3: 3-D Protein Structure of 30s ribosomal protein obtained from PHYRE 2 Online Server

Filename	molpdf	DOPE score	GA341 score
query.B99990001.pdb	2433.97314	-5587.38818	0.00284
query.B99990002.pdb	2484.40112	-5688.02393	0.00153
query.B99990003.pdb	2443.63550	-5641.63281	0.00202
query.B99990004.pdb	2535.40601	-5932.34229	0.01988
query.B99990005.pdb	2459.95068	-5488.01855	0.00547
query.B99990006.pdb	2359.66846	-5850.12842	0.00124
query.B99990007.pdb	2485.16797	-5853.56104	0.01223
query.B99990008.pdb	2395.85303	-5383.38721	0.00625
query.B99990009.pdb	2525.85327	-5654.94775	0.00750
query.B99990010.pdb	2522.07251	-5392.26611	0.00411

Figure 4: Dope Score of the Final Selected Templates Obtained from the Results of PHYRE 2 and 3DPSSM Online Servers (Marked template is selected for loop modeling)

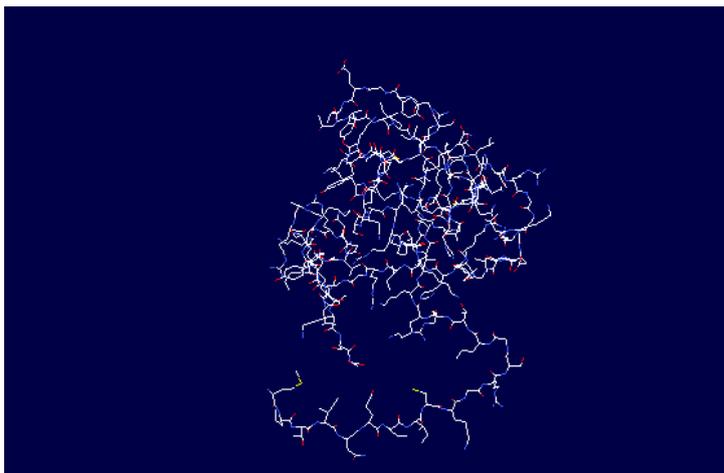


Figure 5: Three Dimensional Structure of Template Protein Sequence Selected for Loop Modeling

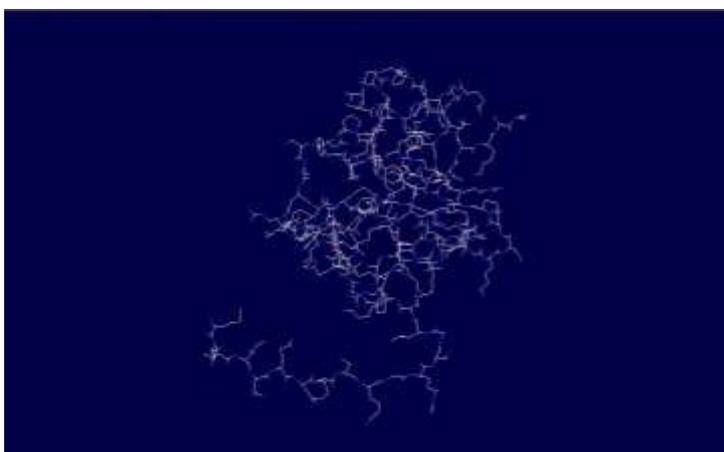


Figure 6: Final Modelled Protein Structure Obtained After Verification

Procheck		
Summary		
1	Note	Ramachandran plot: 96.0% core 4.0% allow 0.0% gener 0.0% disall [PostScript] • [PDF] • [JPG]
2	Warning	+ All Ramachandrans: 1 labelled residues (out of 122) [PostScript] • [PDF] Images: 1 2 3
3	Warning	+ Chi1-chi2 plots: 1 labelled residues (out of 68) [PostScript] • [PDF] Images: 1 2
4	Note	Main-chain params: 6 better 0 inside 0 worse [PostScript] • [PDF] • [JPG]
5	Note	Side-chain params: 5 better 0 inside 0 worse [PostScript] • [PDF] • [JPG]
6	Error	* Residue properties: Max.deviation: 2.3 Bad contacts: 5 * Bond len/angle: 5.2 Morris et al class: 1 1 4 G-factors Dihedrals: -0.16 Covalent: -0.50 Overall: -0.27 [PostScript] • [PDF] Images: 1 2
7	Note	G-factors Dihedrals: -0.16 Covalent: -0.50 Overall: -0.27 [PostScript] • [PDF] • [JPG]
8	Note	M/c bond lengths: 97.0% within limits 3.0% highlighted [PostScript] • [PDF] Images: 1 2
9	Note	M/c bond angles: 85.9% within limits 14.1% highlighted [PostScript] • [PDF] • [JPG]
10	Warning	+ Planar groups: 97.2% within limits 2.8% highlighted [PostScript] • [PDF] • [JPG]

Figure 7: Ramachandran Plot Details of Final Modelled Protein obtained from SAVES (PROCHECK Analysis)

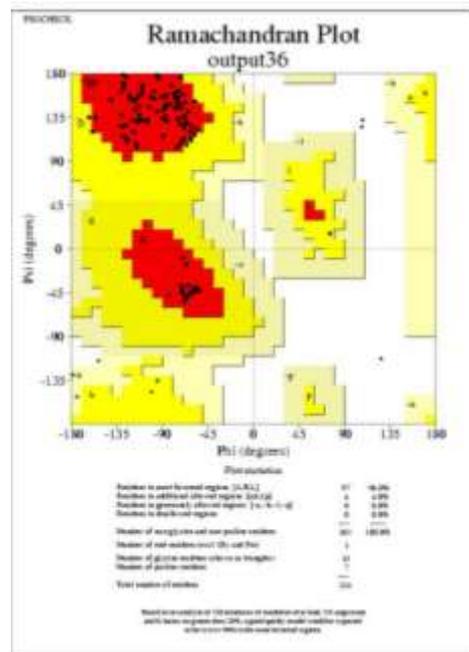


Figure 8: Ramachandra Graph of the Final Modeled Protein obtained from SAVES Online Server