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Insilco Analysis of *Wolbachia Surface* Protein in *Wolbachia Endosymbiont* of *D. Melenogaster*

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ABSTRACT

Wolbachia surface protein is a gram negative bacterial membrane protein which participates in host immune response, pathogenesis and controlled cell death programme. The surface protein of the bacterium is highly important because it acts a source for electing strong immunogenic role and development of vaccines. Bio-physical characterization of *Wolbachia* surface protein is of utmost importance as it would pave way for novel drug designing. The current study employs the bio-informatics tool to unravel the structural and functional properties of the protein. The WSP was predicted to be a β barrel transmembrane protein. The structure was predicted by Homology modeling and Validated by Procheak. The Investigations on WSP in target template sequence alignment reveled 34% homology to Neisseria surface protein (NSP A) and the superimposition of the modeled protein showed highest similarity with OMP of E coli which act as porins; it has shown to work as a channel to transport small compounds across the planar lipid bilayer.

Introduction:

Wolbachia discreet itself from rest of the infectious symbiotic bacteria by being the most abundant life forms infecting over [1].The bacterium is maternally inherited and sustain itself in arthropod host species by inducing a variety of reproductive alterations such as parthenogenesis, feminization and Cytoplasmic incompatibility(embryonic lethality in crosses between infected males and uninfected females)[2].Besides causing female biased sex distortion, the parasitic association of *Wolbachia* can also be mutalistic. For example in Brugia malayi, the nematodes which cause Lymphatic filariasis, Elephantiasis and African river blindness in humans and animals are so much dependent on *Wolbachia* that in the absence of *Wolbachia* dramatic effects can be observed such as delayed moulting, reduced growth rates, aberrant embryogenesis and eventual death [3].

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The comparative genome analysis of *Wolbachia* and other endosymbionts have revealed interesting facts for investigating the role of these bacteria. *Wolbachia* have small genomes only about million base pairs. They lack genomic stability when compared with other obligate endosymbionts[4].Unlike other endosymbionts; *Wolbachia* genome contains high number of repetitive elements (ANK domains) and surface proteins. The genetic distinctiveness of different *Wolbachia* strains is being extensively characterized by these genes[5]. The molecular mechanism of these interactions has not been characterized so far. A key feature observed for such anomalies might be because of *Wolbachia* undergoing extensive recombination.

Several theories and experimental evidences are available to shown that recombination is a major force that can bring about diversity and adaption[6]. The phyogenetic characterization of ankyrin proteins, surface proteins and some hypothetical proteins has supported the hypothesis of extensive recombination in *Wolbachia*. The divergence of WSP gene is (more than 43%) compared with ankyrin (30%), other housekeeping genes (9%)[7, 8]. Apart from such high divergence WSP is undergoing positive selection[9]. The sites of WSP undergoing positive selection lie outside the transmembrane region and hence are quite naturally exposed

to host. By examination on sequences of *Wolbachia* super groups it is known that WSP consists of four Hyper Variable Regions (HVRs) separated by conserved regions[10]. These HVRs undergo high rate of intra and intergenic recombination. HVRs evolve at a faster rate than other genes in the arthropod *Wolbachia* genome and have undergone strong positive selection [9, 10, 11]. It may hypothesize that the positive selection is favored due to HVR interacting host factors. So further investigations are needed to identify the role of *Wolbachia* surface protein gene in arthropods, where *Wolbachia* are known to have a parasitic association..

WSP (*Wolbachia* surface protein) is one of the most translated membrane protein of *Wolbachia* present in Drosophila host. It is a low-molecular-weight protein of 22 kDa[12]. WSP belongs to pfam0617, primarily defined by antibody recognition[13]. Pfam01617 proteins belong to a larger group (CL0193) of beta-barrel protein families with varying numbers of beta strands. So far several *Wolbachia* proteins have been proposed to be involved with the reproductive distortions of the infected hosts[14, 15]. However, no conclusive evidence is available as no proteins satisfactorily answers the raising questions on the host Reproduction alterations. Adding up to this is the variability observed between WSP sequences of different *Wolbachia* strains suggesting that this gene could be used in predicting reproductive phenotypes generated by different strains[16].

Although an insight of homology modeling as already predicted[17] but the detailed analysis of the physiochemical properties, Signal peptide prediction, Subcellcular localization and other structural details was not provided. The study would provide structural details and other properties which are very much necessary in understanding the *Wolbachia* -host interactions with special reference to parasitic *Wolbachia* which causes different reproductive anomalies in arthropod hosts. The structural characterization would provide the clues to its biological function, physiological role and is a prerequisite for the development of new drug targets.

Materials and Methods:

Sequence Selection:

The WSP protein sequence of *Wolbachia* in D.menalogaster (NP_966785.1) is selected from SWISSPORT database. (http://web.expasy.org/)

Physiochemical analysis:

Theoretical PI and Molecular weight was determined using PROTPARM server (http://web.expasy.org/protparam/)

Transmembrane region prediction:

The transmembrane beta strands of WSP were predicted using the web server Pred TMBB (http://biophysics.biol.uoa.gr/PRED-TMBB/)[18].

Sub cellular Localization:

The Sub cellular localization of the WSP protein was predicted using the tool Target P [19].

Signal peptide prediction:

The signal peptide present in WSP was predicted by SignalP 3.0 server (http://www.cbs.dtu.dk/services/SignalP/)

Target template Alignment:

The protein was modeled by aligning between WSP (Target) and NspA (Template). The NCBI Protein BLAST of WSP protein (target) was done against PDB database to obtain a structurally similar protein i.e. template. The wsp sequence alignment was done using CLUSTAL W. The resulting alignment file was provided to SWISS MODEL sever (http://swissmodel.expasy.org/) with the alignment input format CLUSTAL W to obtain a 3D model of the protein.

Homology Modeling:

The three dimensional structure of WSP protein was modeled using MODELLER software using homology modeling. The protein was modeled based on alignment between target and template. NCBI Protein BLAST of WSP protein (target) was done against PDB database to obtain a structurally similar protein i.e. template. The target template sequence alignment was done using CLUSTAL W. The resulting alignment file was provided to SWISS MODEL server (http://swissmodel.expasy.org/). With the alignment input format CLUSTAL W to obtain a 3D model of the protein.

Structure validation:

The modeled WSP Structure was used for structure validation using SAVS (Structure validation and analysis server). The PROCHEAK was used to check the stero chemical quality of the protein structure producing a number of post script plots analyzing its residue by residue geometry.

Results and Discussion:

Annotation of protein function is one of the key problems in post genomic era. This demands bioinformatics and computational biology to predict the function of unannoated hypothetical proteins by using various efficient tools and web servers. In our study, the sequence and structural features of WSP and its complexes is annotated. The results are discussed under following heads

Physiochemical analysis:

The Physiochemical analysis showed WSP had 237 amino acids with a molecular weight of 25450.5 daltons. The protein had an isoelectric point of 4.83. The chemical formula of protein was C1162H1750N280O357S3 indicating total number of 3552 atoms. The extinction coefficient measured at 280nm was found to be 28310, assuming all cys residues as half cystines the absorbance of 1.112. (Abs 0.1% 1g/l). The instability index was computed to be 23. 72 thereby classifying the protein as stable. The aliphatic index is about 76.16 % indicating the protein is thermostable and Grand average hydropathicity is about -0.122.

Transmembrane region prediction:

The Sequence scored a value of 2. 859, which is lower than the threshold value of 2.965. The difference between the value and the threshold indicates the possibility of the protein being an outer membrane protein.

Sub cellular Localization:

The scores obtained prediction of localization; the possible values are tabulated in table 1. Based on the scores obtained prediction of localization for WSP was found to be Secretory pathway, i.e. the sequence contains SP, a signal peptide and Reliability class, 1 indicates the strongest prediction. (See table 1).

Signal peptide prediction:

The signal peptide of WSP was "MHYKKFFSAAALATLLSLSNSAFS" and the cleavage site occurs between pos 24 and 25: AFS –DP .Fig 1 Target – Template Alignment: However, the query coverage between template and target protein sequence is 34%. (Fig.2) Due to the minimal query coverage and identity, the protein sequence was also submitted into Phyre web server (Fold recognition) which shows the same results as BLASTP. From these results, it has confirmed that the crystal structure of Neisseria surface protein A from *Neisseria meningitides* will be used as template for further target – template alignment and model building steps.

Homology Modeling and Structural Analysis of WSP:

The structure function and the location of WSP in outer membrane is unknown. However, infected *Drosophila* eggs express WSP as most abundant protein [12], probably due to strong influence of the protein and its role in host bacterium interactions[20, 21]. In sequence homology, WSP is 34% identical to outer membrane protein NspA from *Neisseria meningitides*, which has been hypothesized as a promising candidate for vaccine development. The NspA shows complete homology with Opa proteins, which mediate adhesion to host cells. In realignment 1P4T & 2U2F doesn't fit properly. The overall modeled structure of WSP is 8-stranded beta-barrel ~60 Å ang long with extended loops exposed towards the extracellular surface. Both N and C-terminus of the modeled structure are towards periplasmic space. The 32 residues on the N-terminus are missing. In the structure the minimum barrel length is 18.7 Å and max 26.4 Å which is similar to the width of porins too (2POR). This thickness is not sufficient to traverse the bacterial lipid membrane ~80 Å thick (Fig 3). However experiments with OmpW, of similar barrel length, the outer membrane protein of E.coli have shown it to work as a channel to transport small compounds across the planar lipid bilayers [22]. In the WSP, the flexible extracellular loops appear relatively more functional than strongly conserved residues in the Beta-barrel. *Wolbachia* being intracellular parasite, the propensity of proteolytic cleavage of extracellular loops will reduce the efficacy of bacterial invasion. The extracellular loops in the homology prediction show an extreme plasticity and a mutational pattern that appears largely unpredictable. The homology modeling showed highest similarity with OMP of E.coli. Single channel conductance experiments in bacterial OmpW shows it as an ion channel in planar lipid bilayers. There are two salt-bridges between R40 -E230 & K83-D130. Tyrosine 38, 128, 141, 187 blocks the channel by making a barrier protected by salt bridge on each side from this we could suspect that there may be channel inside the WSP. Most of the residues in the cavity are charged residues blocking the way, unlike as in porin. So it could be very selective. Generally channels are divided in three classes, based on their mode of transport: general porins, substrate-specific transporters, and active transporters. From the observed three layer blockage in the channel it cannot be a general porin, whereas the WSP being a substrate specific transporter is much higher. To be an active transporter, there should be an energy mediated transportation with an ability to bind energy currency (eg. ATP, GTP etc.). The structural comparison of yielded a homolog of several pfam related proteins when searched using Phyre web server (Fold recognition) which includes OmpA, (Outer Membrane Protein of Anaplasma) assisting the invasion of the bacteria into the host cells [23]. Other OMP (Outer Membrane Protein) are known to act as channels and facilitate passive and active transport [24]. These similarities suggest that WSP may have a comparable role in *Wolbachia* interactions with host and WSP may also inhibit apoptosis of host cells.

Structure validation:

The predicted complex structure was observed in PROCHEAK 3D validation showed 84.15% of steriochemical rotation of torsion of angles and no amino acid changes in protein complex.

Conclusions:

In absence of a high resolution structure of WSP so far hindered attempts to predict more specific functions to this protein. The conformation of these requires crystal structure and complete functional description to elucidate the effects of non synonymous substitutions on protein structure and functionality. The over expression, purification of the WSP is underway. The crystal structure of WSP and the complexes will provide insights into the interactions between the two proteins and the logic behind conserved domains between HVR'S will be analyzed. The same methodology will be extended to study other groups of WSP and host proteins. The comparative analysis will highlight the multiple roles of *Wolbachia* proteins which extend to cytoplamic incompatibility, feminization of genetic males, parthenogenesis induction and male killing. Further Structural characterization of WSP will be a break through towards gaining information on *Wolbachia* induced different phenotypes and further this would be used for applied research such as i) Management of (Arthropod) pest that cause major damage in agriculture industry. ii) Control of important vectors that cause the major diseases. iii) Enhancing the fitness and efficacy of Bio control agents. iv) Development of new drug targets.

Fig 1: Signal P NN result for the signal peptide of WSP protein using several artificial neural networks and Hidden markov models. The Signal peptide is present between position 24 and 25 in WSP sequence. Score C, S, and Y represent cleavage site score, signal peptide score and combination score (derived from C and S scores).

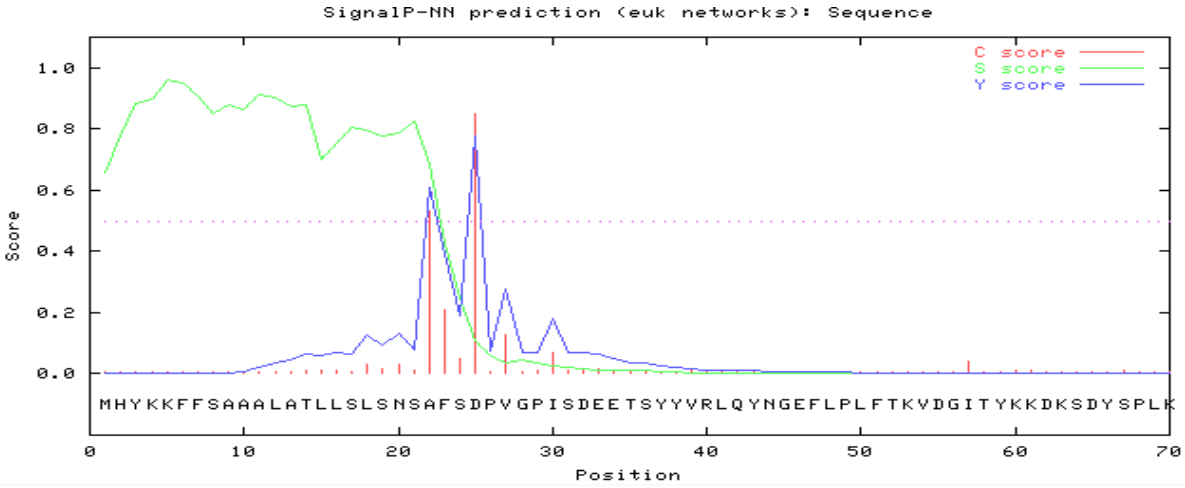

Fig 2: A Sequence alignment between WSP (Target) and Nspa (Template) The template crystal structure of Neisserial Surface Protein A (Nspa) [PDB ID: 1P4T] from Neisseria meningitides showing 34% identity with the given target sequence. From these results, it has confirmed that the crystal structure of Neisseria surface protein A from Neisseria meningitides will be used as template for further target – template alignment and model building steps

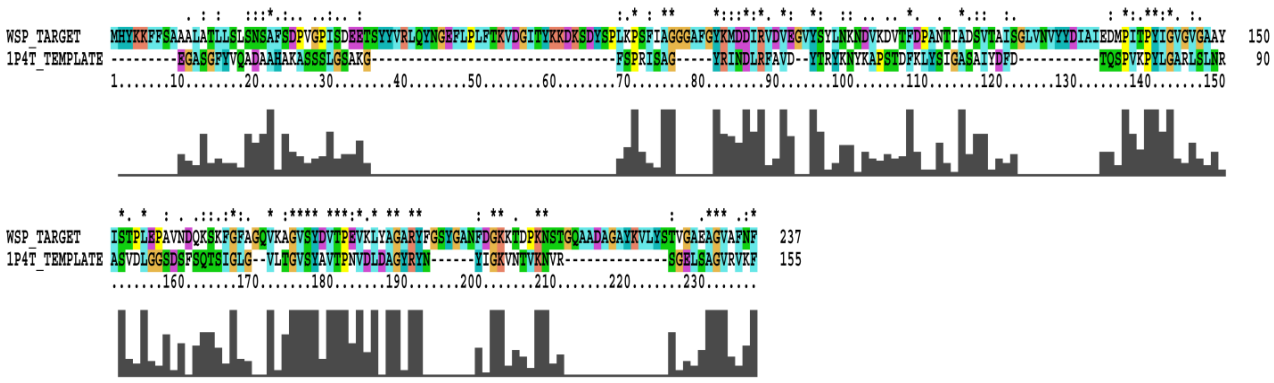

Fig 3: Three dimensional structure of WSP in D.melanogaster showing 8 beta barrel weight of ~80.747 Angstrom (A), length 18.7A and width of 26.4 (A).

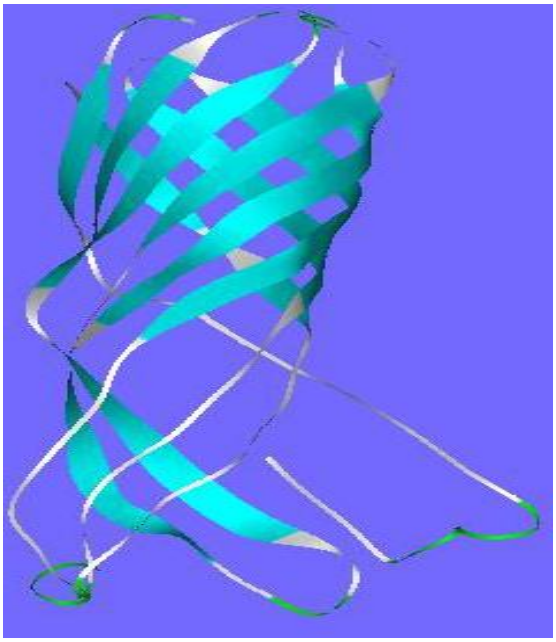

Table 1: The target P prediction for sub cellular localization of *Wolbachia* surface protein showing sequence length 237 and mTP score 0.033 and SP sore of 0.894 classifying this protein as found to be Secretory pathway, i.e. the sequence contains SP, a signal peptide and Reliability class,(RC) 1 indicates the strongest prediction.

Name	Len	mTP	SP	other	Loc	RC
Sequence	237	0.033	0.894	0.080	S	1

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