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Characterization of *Wolbachia* cell division protein (ftsz) gene for potential management of Uzifly *Exorista sorbillans* (Diptera: Tachinidae)

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Abstract
Wolbachia is an intracellular alpha-proteobacteria Rickettisial endosymbiont present in most of the arthropods. *Wolbachia* a parasite in insects invades its host biology in many ways, including cytoplasmic incompatibility, feminization, male killing, parthenogenesis and reproductive fitness advantage. The whole genome sequence of *Wolbachia* reveals the function of several genes like wsp (*Wolbachia* surface protein) FtsZ (*Wolbachia* cell division protein) and ANK (ankarin domain protein). Among these, FtsZ (Filamenting temperature sensitive) protein plays a vital role in the cell division of *Wolbachia* .In the present study, we characterized the *Wolbachia* FtsZ gene infected uzifly, *Exorista sorbillans* a serious menace to silkworm *Bombyx mori* L. The study revealed the possible implications of FtsZ gene to combat the uzifly *E. sorbillans* an endoparasite of *B. mori* L.

Keywords: Control, Cell division. Parasite, Protein, Uzifly, *Wolbachia*.

1. Introduction
Wolbachia a maternally inherited gram negative endosymbiotic bacteria abundantly found in arthropods. It has been estimated that 44% insect species including the arthropods like spiders, mites, isopods, springtails and nematodes are infected with *Wolbachia*. [1-5] *Wolbachia* associated arthropod studies clearly indicated that, *Wolbachia* is profoundly localized in the reproductive tissues of arthropods inducing an array of reproductive manipulations. The reproductive manipulations induced by *Wolbachia* are cytoplasmic incompatability (CI), feminization of genetic males, male killing and induction of thelyotokous parthenogenesis [6-7].
The molecular phylogenetic analysis based on primary sequence information of 16S rDNA genes indicated that *Wolbachia* belongs to alpha-proteobacteria [8]. The *Wolbachia* whole genome sequencing of *Drosophila melanogaster* (*w*Mel) has provided a significant genomic information [9]. Based on the whole genomic information Multi Locus Sequence Typing (MLST) system was developed to know the *Wolbachia* strain [10]. *Wolbachia* infection in arthropods based on the MLST sequencing sub divided in to 10 phylogenetic clades [11]. MLST information of five housekeeping genes of *Wolbachia* namely *coxA, gatB, hcpA, ftsZ* and *fbpA* in addition to *wsp*, is widely accepted method for strain typing [10].
The FtsZ (Filamenting temperature sensitive) protein plays a vital role in cell division in both prokaryotes and eukarytic organisms. FtsZ is the most conserved gene in bacterial cell and the FtsZ protein sequence analysis shown that might have evolved soon after the bacterial cell [12]. FtsZ protein localizes beneath the cell membrane at centre along with the cytoplasm into the contractile Z-ring. For the Z-ring assembly two important dynamic functional factors are required, those are polymerization of the FtsZ monomers into protofilaments and GTPase. *Wolbachia* FtsZ was found to play active role in the life cycle of bacteria [13]. In the present study, we describe the molecular characterization and mechanical properties of *Wolbachia* FtsZ gene of uzifly *Exorista sorbillans* in in-silico modelling and its implications as a possible approach to control uzifly *E. sorbillans* are also discussed.

2. Material and Methods
2.1 Sequence retrieval
The DNA sequences of *Wolbachia* cell division protein (FtsZ) of *Exorista sorbillans*, FtsZ-A, JQ946085 and FtsZ-B, JQ946084 were retrieved from NCBI. All the sequences were subjected to translating in to protein sequence

2.2 Secondary structure prediction
Computational methods were used to predict secondary structure of protein using SOPMA and PHYRE program. For SOPMA analysis, primary sequence of protein was submitted to program. The above said programs provided detailed information on structures of Helices, coils and strands of FtsZ protein. The secondary structure prediction of protein are analyzed by SOPMA are predicted 65.5% are good secondary structure [14]. By using PHYRE the secondary structure of protein sequence was analyzed.

2.3 Physico chemical properties
Amino acid sequence was used here to predict the physicochemical properties of using ProtParam. By using physicochemical properties, we can predict the stability of protein structure. Instability index value should be below 40 is structure is stable, above 40 leads to structural instability [15].

2.4 Homology modelling
Automated model building program I-TASSER was used to predict 3D model of protein. Protein sequence was used to submit to ITASSER to predict the 3D structure. It utilizes the templates to build by using protparam structure of protein target. Templates selected based on Template Modeling Score (TM-score-is an algorithm used to calculate the likeness of topologies of two protein structures) were used to predict 3D structure. Based on these templates ITASSER predicted five models computationally by using C-score algorithm value -5 to 2, C-score represent the confidence score of model and if C-score increases, confidence of the model too increases.

2.5 Ramachandran
Plot analysis phi and psi angle was done using the RAMPAGE analysis program to check Phi and Psi angle of protein structure.

JQ946085
MLVKVQQKNQLMKLWSIKIVICSLSQQGWVVVLEQ VLHRLQRQPEKQERLKIREQKKKRYLLELLSRSVLK VCDVCALQSLDLKSCKNTIHLLSFPIKIYLELLTRKL HLLTHFNSPIMFCILAEELISCQDLILILLIKQVRWVK QLVLERQKEKIGQLVLQRLRYLIHCLTMYQKVRKE YLILLVVETLYLKLILQPIECVKKWMKMQIYLVPLLI RL

3. Results
3.1 Sopma and Phyre results
SOPMA Analysis
FtsZ-A protein secondary structure are listed in table-1 shows structure of alpha helix and follows extended strand and Random coil share same percent of structure in the FtsZ protein. Secondary structure prediction by **SOPMA** was done by taking default parameters (Window width: 17, similarity threshold: 8 and number of states: 4).

Table 1: Calculated secondary structure elements by SOPMA.

Sl.no.	Contents	Value (%)
1	Alpha helix	72.40
2	3_{10} helix	0.00
3	Pi helix	0.00
4	Beta bridge	0.00
5	Extended strands	12.22
6	Beta turn	3.17
7	Bend region	0.00
8	Random coil	12.22
9	Ambiguous states	0.00
10	Other states	0.00

PHYRE analysis
Secondary structure disorder prediction was done by **PHYRE** program as showed in Figure -1. Secondary structure and its disorder shows 7% is disorder, 92% is Alpha helix and 1% beta strand.

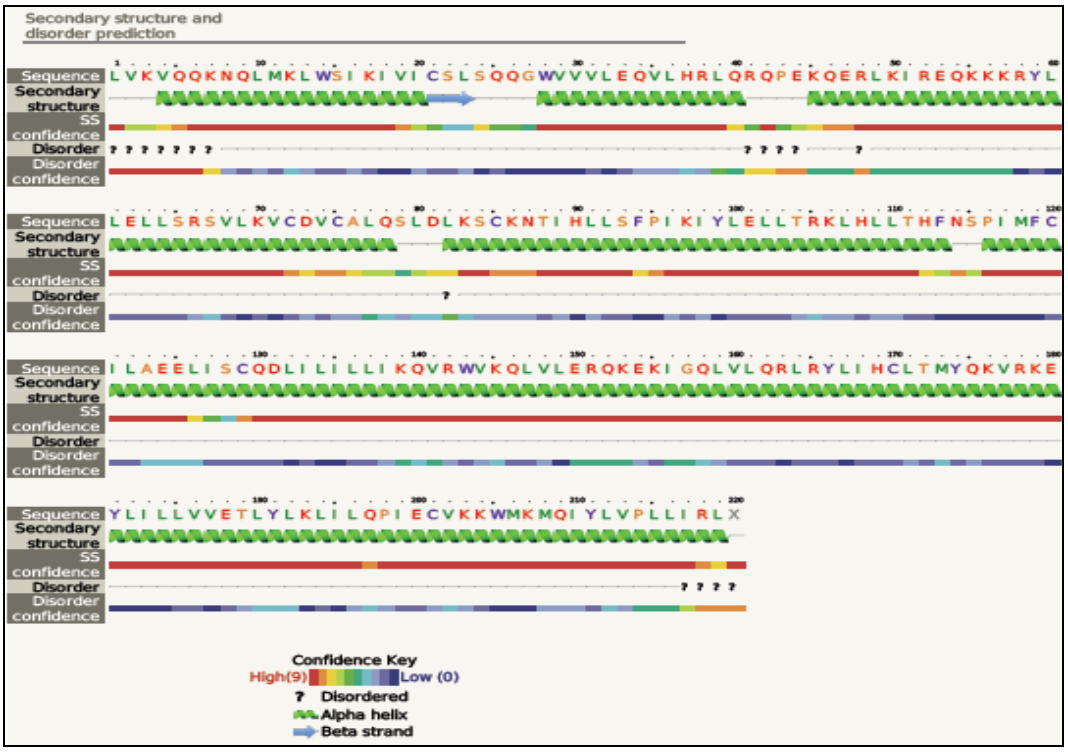

Fig 1: Secondary structure of *Wolbachia* ftsZ-A protein predicted by Phyre 2

3.2 Physico chemical properties by ProtParam

Our predicted results from ProtParam analysis showed FtsZ protein instability index (II) was 51.98. It was showed FtsZ protein is unstable. Extinction coefficient was 33055, showed low frequencies of CyS residues. Aliphatic index value 145.18, indicated thermal stability. The high value of aliphatic index predicted that protein is stable in high thermal conditions. Another Grand Average Hydropathy (GRAVY) value was 0.284, which indicates interaction with water.

3.3 Modeling

We have chosen best CN A model-1 has C-score -4.19 among the 5 suggested models based on C-score of generated models as follows as, model-2 C-score=-4.49, model-3 C-score=-4.77, model-4 C-score=-4.96 and model-5 C-score=-5 Figure-2. [16]

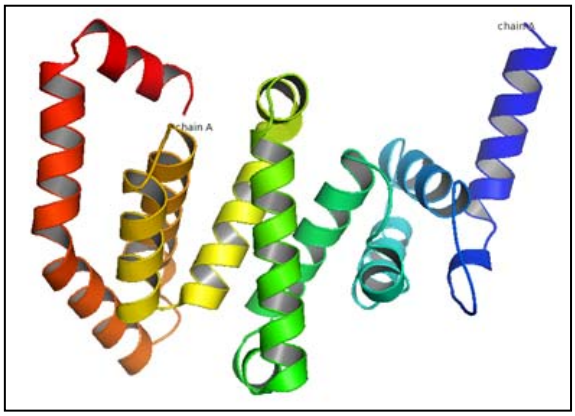

Fig 2: Ribbon model of *Wolbachia* ftsZ-A protein modelled by ITASSER.

3.3 RAMPAGE analysis

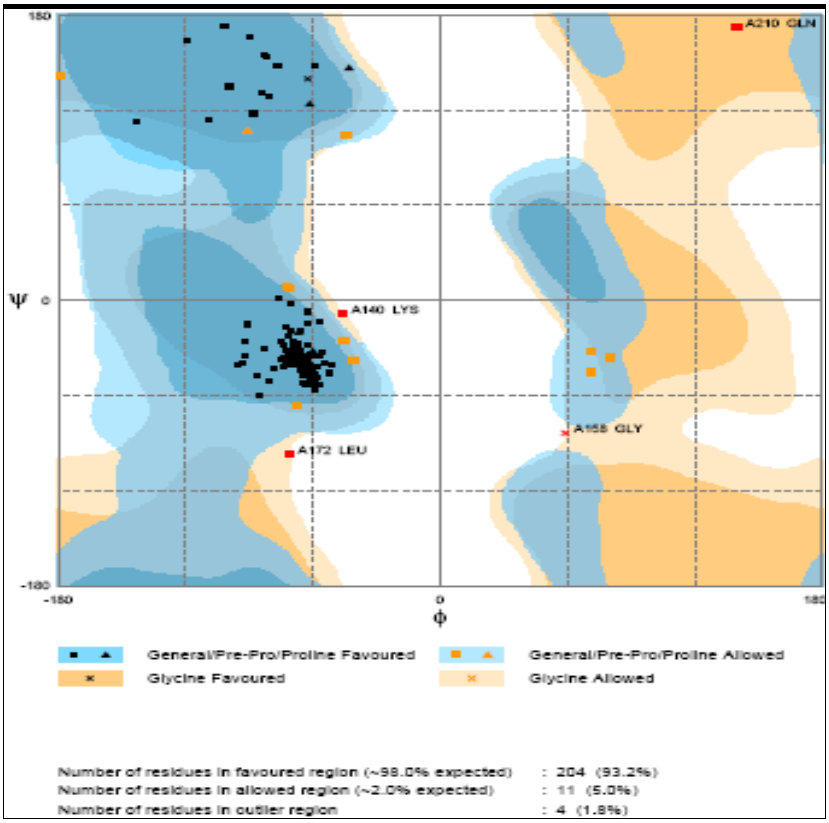

Fig 3: Analysis of Ramachandran plot analysis of modeled protein *Wolbachia* ftsZ-A by RAMPAGE.

Ramacahndran plot analysis results confirm the stability of protein shows 93.2% of total residues are fall in most favoured region, 5% of total residues are allowed region and least 1.9% resides fall in outer region (Figure-3). This result shows modelled protein backbone dihedral Phi and Psi angle are in precise position [17].

JQ946084
MLAKVQQKSQLMRLWSIKIVICFSSQQEWAVVLEPV QHRLQKQPEKQEPQLRIERQKKKRYLLELLNRSVL KVCAVCALQSLDLKNCKNTWIHLLSFQIRIYLELQM KKLHFLMHLNLLIMFCTLASEELTWSCQGLSILTSLI KQARWAKRSAPERQKEKIEQLVLQRLQYLIHCLIM YQKVRKEYLTLLVAEILCLKLMLQPIECVKKMKMQ IYLVLLLIKRWREDC

3.4 Sopma analysis

FtsZ-B Protein secondary structure are listed in table 2 shows more structure is alpha helix follows Random coil share grater percent of structure in the protein than Extended strand and beta turns. Secondary structure prediction by **SOPMA** was done by taking default parameters default parameters (Window width: 17, similarity threshold: 8 and number of states: 4).

Table 2: Calculated secondary structure elements by SOPMA.

S.no.	Contents	Value (%)
1	Alpha helix	75.98
2	3_{10} helix	0.00
3	Pi helix	0.00
4	Beta bridge	0.00
5	Extended strands	7.86
6	Beta turn	4.37
7	Bend region	0.00
8	Random coil	11.79
9	Ambiguous states	0.00
10	Other states	0.00

3.5 PHYRE analysis

Secondary structure disorder prediction were done by PHYRE program are showed in Figure-4. Secondary structure and its disorder are results shows 13% is disorder, 85% is Alpha helix and 7% beta strand.

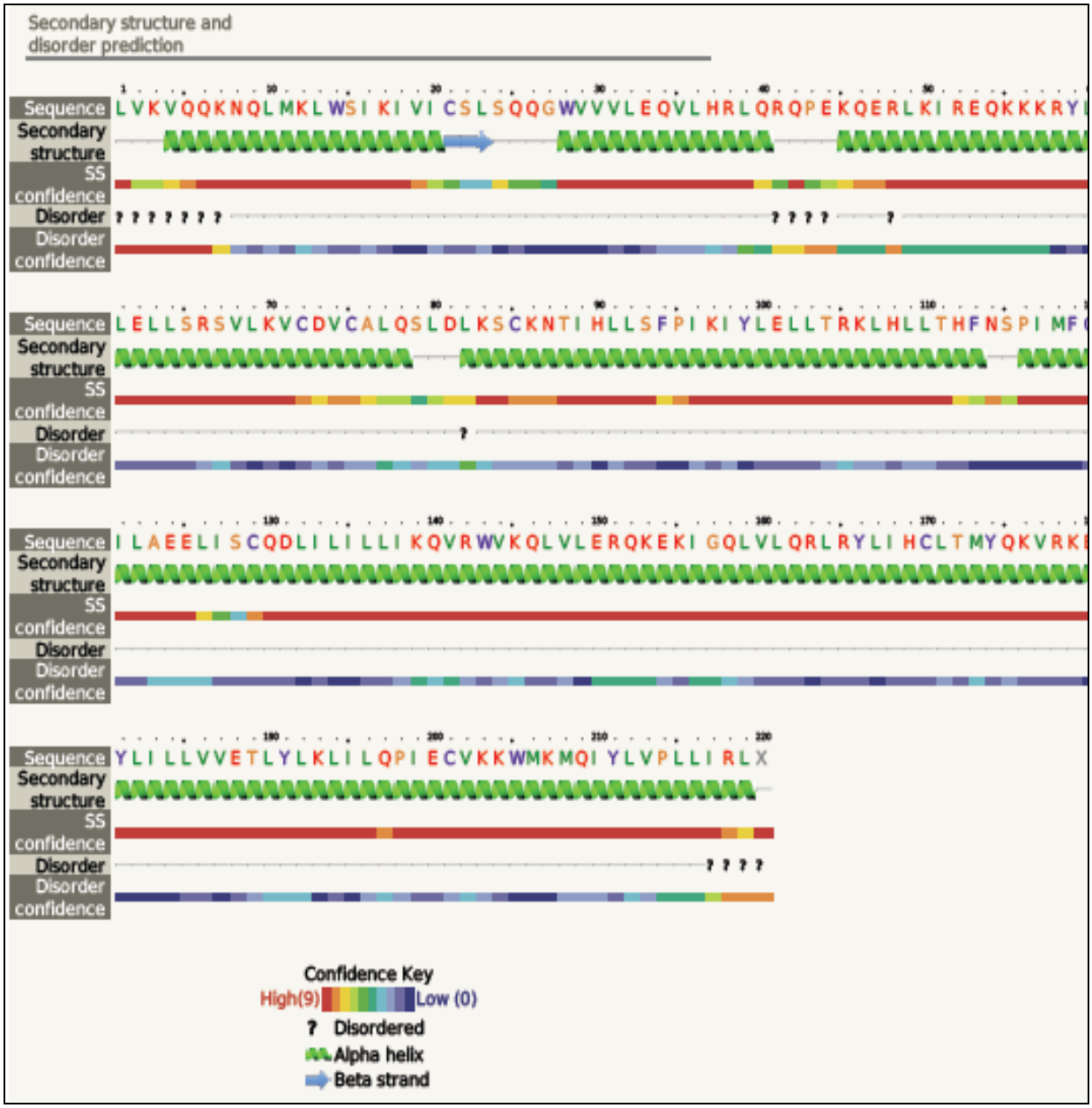

Fig 4: Secondary structure of *Wolbachia* ftsZ-B protein predicted by phyre2

3.6 Physico chemical properties

Our predicted results from ProtParam analysis shows protein instability index (II) is 68.09. It shows this protein is unstable. Extinction coefficient 41940 shows low frequencies of CyS residues. Aliphatic index value 124.28 indicates the thermal stability. This high value of aliphatic index predicts protein may be stable in high thermal conditions. Another Grand Average Hydropathy (GRAVY) values is 0.013 indicates affinity with water.

3.7 Modeling

We have chosen best CN A model-1 has C-score -2.40 among the 5 suggested models based on C-score of generated models as follows as model-2 C-score=-4.12, model-3 C-score=-4.34, model-4 C-score=-5 and model-5 C-score=-5 Figure-5.

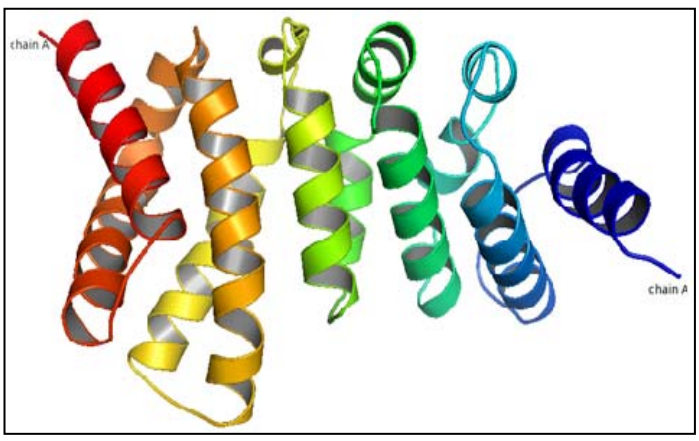

Fig 5: Ribbon model of *Wolbachia* fts Z-B protein modelled by ITASSER

3.8 RAMACHANDRAN plot analysis

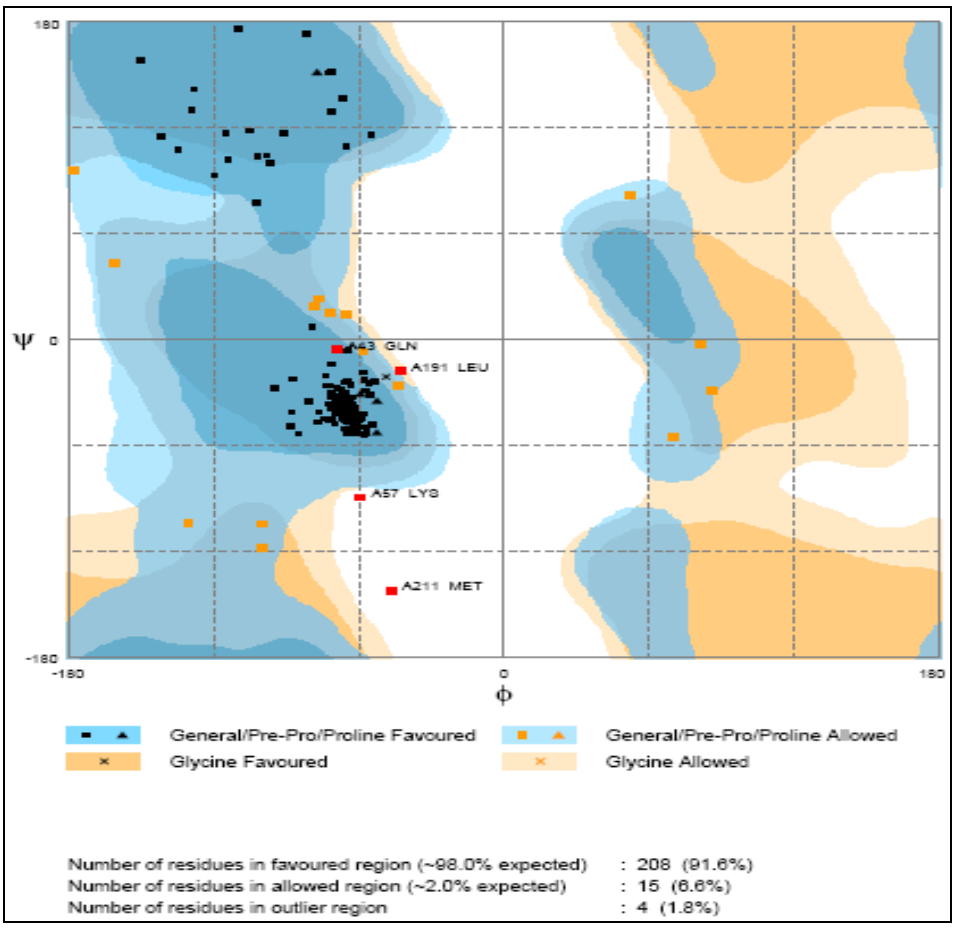

Fig 6: Ramachandran plot analysis of modeled protein *Wolbachia* fts Z-B by RAMPAGE.

Ramachandran plot analysis results confirm the stability of protein shows 91.6% of total residues are fall in most favoured region, 6.6% of total residues are allowed region and least 1.8% residues fall in outer region (Figure-6). This result shows modelled protein backbone dihedral Phi and Psi angle are in precise position.

4. Discussion

Filamenting temperature sensitive (FtsZ) protein is prokaryotic specific cell division essential protein widely conserved in bacteria. Ftsz protein is mainly involved in cell division and cell wall synthesis of bacteria. FtsZ is the most primitive widely conserved protein in bacteria that might have developed rapidly following the cell evolution [18]. The cell division amide before the FtsZ localizes beneath the cell membrane to form Z-ring [19]. Z-rings have functional properties like polymerization of FtsZ monomers in to protofilaments and GTPase activity. Inhibition of these two phenomena through the antibacterial compounds is lethal to bacteria [20].

In the present study, we modeled the FtsZ protein of *Wolbachia* endosymbiont of uzifly *E. sorbillans* a serious menace to silkworm *B. mori*. Our earlier results clearly revealed the existence of a strong direct benefit of *Wolbachia* infection for females as previously described [21]. With the elimination or hindering of *Wolbachia* by antibiotic therapy, there is a drastic reduction in fecundity and hatching of uzifly. This may be due to the inhibition of FtsZ protein (*Wolbachia* cell division protein) by antibacterial compounds which in turn may have larger effect on the population structure.

In summary, the *Wolbachia* cell division protein FtsZ plays a vital role in the cell division and biology of the uzifly *E. sorbillans.* Use of FtsZ cell division protein through inhibitor compounds like antibiotics inhibit the activity of cell division and is a novel therapeutic method to control uzifly a serious menace of silkworm *B. mori*.

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