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Research Article

Structural Analysis of Major Translocator-Chaperone Interaction from Ysa-Ysp Type III Secretion System of Yersinia Enterocolitica - a

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ABSTRACT

YspB is a major translocator protein of *Yersinia* secretion apparatus- *Yersinia* secretion protein (Ysa-Ysp) Type III Secretion System (T3SS) of *Yersinia enterocolitica* Biovar 1B. SycB is the cognate class II chaperone of YspB. YspB is a highly alpha helical protein. It shows significant homology to IpaB-SipB family of proteins. YspB possesses transmembrane helices, intramolecular coiled-coil regions and intrinsically disordered regions, all characteristics of translocator proteins. Homology model of YspB showed an all helical structure interspersed by coiled regions. YspB has a star shaped three dimensional structure with five distinct arms. The first two Tetratricopeptide Repeat (TPR) regions of SycB are responsible for its interaction with YspB. The helices and the loops of YspB interacting with SycB exhibit evolutionary conservation. Besides this, some structurally conserved amino acid residues were also observed in other helices and loops of YspB. The nature of residues involved in the YspB-SycB interaction indicate towards an ionic or polar interaction between the two proteins. This model of translocator-chaperone interaction might prove to be potentially beneficial in understanding the regulation of Ysa-Ysp T3SS.

Keywords: Ysa-Ysp T3SS; Translocator protein; Consurf analysis; Homology model; Molecular docking; Translocator-chaperone interaction

INTRODUCTION

Yersinia pseudotuberculosis, Y. enterocolitica, and *Y. pestis* are the three species of *Yersinia* pathogenic to humans. *Y. pseudotuberculosis* and *Y. enterocolitica* cause gastrointestinal and enteric diseases in humans, whereas *Y. pestis* causes pneumonic and bubonic plague. *Y. enterocolitica* is an opportunistic gram negative bacterium, which causes nosocomial infections and infections associated with wounds and burns in immune-compromised individuals. Yersiniosis is the typical infection caused by *Y. enterocolitica*. Healthy individuals show gastroenteritis and mesenteric adenitis as a result of *Y. enterocolitica* infection. Whereas immune-compromised adults show mortality rate up to 50% due to bacteraemia following gastroenteritis. Children under the age of 5 years form the most vulnerable group and almost half of all the *Y. enterocolitica* infections occur in children of this age group. *Yersinia* shows a widespread distribution in nature including intestinal tract of numerous mammals and avian species as its habitats. Cattle, deer, pigs, birds and humans are frequently infected by this zoonotic pathogen [1-6].

Yersinia enterocolitica possesses Type III Secretion System (T3SS) which function as a nanosyringe and injects bacterial toxic effector proteins into the host cell cytoplasm. Other gram negative bacteria like *Salmonella typhimurium, Shigella flexneri, Escherichia coli* and *Pseudomonas aeruginosa* also possess T3SS as an adaptive feature in them [7]. There are two different T3SS in *Y. enterocolitica*. The genes encoding *Yersinia* secretion component- *Yersinia* outer protein (Ysc-Yop) T3SS exist in a 70 kb pYV plasmid, whereas genes encoding *Yersinia* secretion apparatus- *Yersinia* secretion protein (Ysa-Ysp) T3SS occur in a specific region in the bacterial chromosome. This region within the bacterial chromosome is flanked by direct repeats and is known as Pathogenecity Island. Some of the serotypes of *Y. enterocolitica* Biovar 1B like O:4, O:8, O:13 and O:21 possess Ysa-Ysp T3SS. The two T3SS of *Yersinia enterocolitica*, Ysc-Yop T3SS and Ysa-Ysp T3SS are responsible for the two phases of *Y. enterocolitica* infection. Ysa-Ysp T3SS is responsible for the gastrointestinal phase of infection, which occurs due to the colonization of the pathogen in the gastrointestinal tract of the body [8-11]. The systemic phase of the infection begins when the parasite multiplies in the visceral organs like liver, spleen and lymph node, after evading the M cells on the Peyer's patches of the intestine. Ysc-Yop T3SS is responsible for the systemic phase of infection and evasion of host immune responses [12,13].

The complex regulon T3SS is formed by five different categories of proteins. These are structural proteins, translocator proteins, regulators, chaperones and toxic effector proteins. The components of T3SS are arranged in a sequential manner. First the basal body is formed, followed by the formation of needle substructure by oligomerization of needle forming protein PscF in *Pseudomonas* and YscF in *Yersinia.* Finally, the translocator proteins organize to form the functional translocon on the tip of the needle. The basal body spans the inner membrane, periplasmic space and outer membrane of the bacteria. It anchors the needle to the bacterial membranes. Within the inner membrane, the basal structure ring is made up of lipoprotein YscJ in *Yersinia* and PscJ in *Pseudomonas*, whereas the outer membrane ring of basal structure is composed of secreting YscC and PscC in *Yersinia* and *Pseudomonas,* respectively. The export apparatus forms a platform for the assembly of basal structure. The export apparatus controls the specificity of secreted substrates. The highly conserved integral membrane proteins YscR, YscS, YscT, YscU and YscV and PscR, PscS, PscT, PscU and PscV form the export apparatus in *Y. enterocolitica* and *P. aeruginosa,* respectively. The energy for the transport of toxins comes from the ATPases YscN and PscN, which interacts with YscL and PscL in *Yersinia* and *Pseudomonas*, respectively, just beneath the basal structure [14-22]. The needle complex is needed for the transportation of substrates from bacterial cytoplasm to the extracellular environment. The translocon is important for the secretion of toxic effector proteins into the host cell. The hydrophilic translocator protein (V antigen) PcrV in *Pseudomonas* and LcrV in *Yersinia* oligomerizes to form the platform for the assembly of hydrophobic translocator proteins. Hydrophilic translocator proteins remain exposed to the external environment, which makes them potential therapeutic targets for vaccination. The major hydrophobic translocators PopB in *Pseudomonas* and YopB and YspB in *Yersinia* possess two transmembrane regions, an N-terminal coiled coil region and occasionally, a C-terminal amphipathic helix. PopD in *Pseudomonas* and YopD and YspC in *Yersinia* are the minor hydrophobic translators consisting of a single transmembrane region and occasionally, a C-terminal amphipathic helix. The hydrophobic and hydrophilic translocator together form the outermost part of the needle complex, thereby interacting with the host cell membrane [15,16,19,20-22].

Regulator protein LcrG in *Yersinia* and PcrG in *Pseudomonas* binds to and regulate the secretion of hydrophilic translocator protein LcrV and PcrV in *Yersinia* and *Pseudomonas*, respectively [22-24]. Chaperones of T3SS sequester their cognate binding partners and facilitate their secretion. Chaperone proteins are classified into three categories. Class IA chaperone SpcU binds to a single effector protein ExoU. SpcS is a class IB chaperon from *P. aeruginosa* which

binds to two effector proteins ExoS and ExoU. Class II chaperone PcrH in *Pseudomonas* and SycB in *Yersinia* bind to two different translocators PopB and PopD and YspB and YspC, respectively. Class III chaperones PscE and PscG bind to the needle forming protein PscF in *Pseudomonas*. Chaperones are important for maintenance of their cognate translocators or effector proteins in secretion competent state within the bacterial cytoplasm prior to secretion and facilitate their secretion upon receiving proper activation signals [15,25,26].

syc-ysp operon consists of genes like SycB, YspB, YspC, YspD, YspA and AcpY. This operon is located downstream to genes encoding Ysa-apparatus in ~ 200 kb Pathogenecity Island. SycB is a class II chaperone and binds to major hydrophobic translocator YspB and minor hydrophobic translocator YspC in *Yersinia*. Being a hydrophilic translocator YspD participate in the formation of platform for organization of hydrophobic translocators. YspA is a unique protein. The proteins of Ysa-Ysp T3SS YspB, YspC, YspD and SycB are homologous to SipB, SipC, SipD and SicA from SPI-1 T3SS of *Salmonella enterica*, and IpaB, IpaC, IpaD, and IpgC from Mxi-Spa T3SS of *Shigella flexneri*, respectively. Activation of *YsaE* promoter mediates the transcription of structural genes *sycB* followed by *yspB, yspC, YspD and YspA*. YsaE and SycB interact with each other to give an additive effect on the transcription [10,11,21,27,28]. Ysa-Ysp T3SS is activated at low temperature and high salt conditions. This activation is regulated by proteins YsrR, YsrS, YsrT and RcsB [29-32]. Class II chaperone SycB have a helical structure interspersed by coiled regions. It exists as physiological dimer. The Tetratricopeptide Repeats (TPRs) are formed by the antiparallel alpha helices. These TPRs possess conserved residues in the canonical positions, which participate in protein-protein interaction [33]. The structural characterization of YspB by any biochemical and biophysical method has not been possible yet, as the protein is recalcitrant to recombinant expression. Therefore, we have adopted an in silico approach to decipher the structure of YspB and elucidate the structural basis of YspB-SycB interaction.

MATERIALS AND METHODS

Secondary structure prediction and Multiple sequence alignment in YspB

JPred4 and PsiPred secondary structure prediction servers were used for determination of alpha-helix, beta-sheet and random coil content of YspB [34,35]. Multiple Sequence Alignment (MSA) of YspB with other major translocators like IpaB and SipB was done using MultAlin interface and was edited in ESPript, MSA editor [36].

Prediction of Transmembrane regions, Intramolecular coil coiled regions and disordered regions in YspB

Transmembrane region in YspB was predicted using TMHMM server v.2.0 [37]. Intramolecular coil-coiled region within YspB was predicted by COILS server [38]. Intrinsic disordered region in YspB was determined by disordered prediction servers like PrDOS and DisEMBL 1.5 [39,40].

Homology model of YspB

The homology model of YspB was generated by I-TASSER server using a threading approach [41]. The structural templates were identified by LOMETS from PDB library. The best threading templates selected for YspB were PDB Hits- 4uxyA, lst6A, 3u0cA, 3jbhA, 4hnwA and 4ui9O. Additional restraints were not imposed by the user. The best model was selected based on C-Score, TM-Score and other parameters. The output PDB file of the model was represented.

Dynamic Light scattering

Protein solution was prepared at a concentration range of 2–4 mg/ml in 25 mM Tris-HCl, 50 mM NaCl buffer. Just before the analysis, sample was filtered with 0.22 micron filter to remove any aggregates or particles in the sample and analysis was done in Malvern Zetasizer nano ZS DLS instrument with associated software.

Molecular Docking of YspB and SycB

Z DOCK server (version 3.0.2) was used to perform Molecular Docking of YspB and SycB [42]. The homology model of YspB was loaded as input protein 1 and homology model of SycB was loaded as input protein 2. From the output files the best model of interaction between YspB and SycB was selected.

ConSurf Analysis

The PDB file of YspB model was loaded into ConSurf. MSA was built using MAFFT. Homolog search algorithm was HMMER and homologs were collected from UNIREF90 database. HMMER E-value was 0.0001. Maximal % ID between the sequences was 95, whereas the minimal % ID for the homologs was 30. 150 sequences sample the list of homologs to the query. Phylogenetic tree was constructed using Neighbor Joining with ML distance and Bayesian method was used for calculation of conservation score [43].

Representation of Models

The homology model of YspB and SycB were represented using PyMOL Molecular Graphics System [44]. The model of YspB-SycB generated by Z DOCK server was presented in PyMOL Molecular Graphics System [44]. The model of YspB along with the conservation colour codes generated by ConSurf was represented by UCSF-Chimera [45].

RESULT AND DISCUSSION

Secondary Structure analysis of YspB

YspB is a major hydrophobic translocator protein of Ysa-Ysp type III secretion system. We have retrieved YspB sequence from protein database from National Center for Biotechnology Information with Genbank accession no. AAK84113. Since, YspB is recalcitrant to recombinant expression secondary structure prediction servers like JPred and PsiPred were used for prediction of the secondary structural content of YspB [34,35]. Jpred and Psipred are the leading structure prediction servers which predict the secondary structure of proteins based on the protein sequence, using their experimentally validated algorithm. According to JPred, YspB contained 80.17% alpha helix, 2.07% beta sheet and 17.76% coiled structure (Figure 1A, Table 1). Whereas PsiPred predicted 80.6% alpha helix and 19.4% random structure (Figure 1B, Table 1). Both the servers predicted high alpha helical content of YspB, which is consistent with the secondary structural content of major translocator protein.

Homology of YspB with the proteins of IpaB_SipB family

The proteins of Ysa-Ysp T3SS show homology to the proteins of SPI-1 T3SS from *Salmonella enterica* and Mxi-Spa T3SS from *Shigella flexneri*. YspB, YspC, YspD and SycB are homologous to SipB, SipC, SipD and SicA from SPI-1 T3SS and IpaB, IpaC, IpaD and IpgC from Mxi-Spa T3SS. Multiple Sequence Alignment aligns the various homologous protein sequences belonging to a family and determines the identity and similarity between these sequences i.e. the extent of homology. MSA was performed using MultAlin

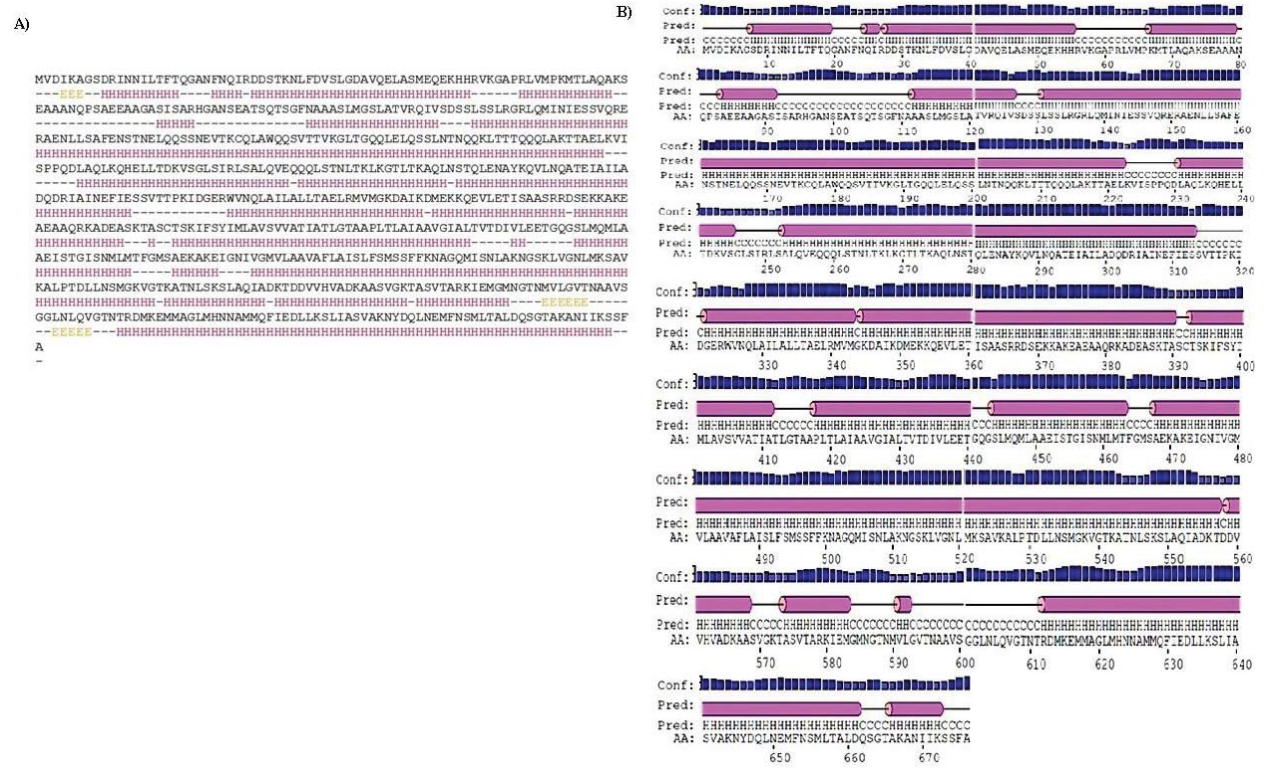

Figure 1: JPred and PsiPred predicted high alpha helical content of YspB: A) Secondary structure analysis by JPred. B) Secondary structure prediction by PsiPred.

Table1: Secondary structure content of YspB predicted by JPred and PsiPred

Secondary Structure / Server Used	Alpha helix	Beta sheet	Random coil
JPred	80.17%	2.07%	17.75%
PsiPred	80.6%	0%	19.4%

interface to determine the homology between YspB and proteins from IpaB-SipB family [36]. YspB shows 14.03% identity, 40.38% similarity, and 87.59% sequence coverage with SipB. Whereas, YspB and IpaB possess 12.85% identity, 39.05% similarity and 85.67% sequence coverage (Figure 2, Table 2). Therefore, translocators from Ysa-Ysp T3SS, SPI-1 T3SS and Mxi-Spa T3SS form one family and translocators of Ysc-Yop, Psc and Asc T3SS form a separate family of translocators based on their homology and sequence conservation.

Transmembrane regions, intramolecular coiled coil regions and disordered regions in YspB

YspB has to traverse the bacterial plasma membrane to reach the translocation apparatus or translocon. It also interacts with the host cell plasma membrane. So, there is a high probability that YspB possesses some transmembrane helices for the aforesaid purpose. TMHMM is a very reliable server which indicates the probability of a protein sequence to form a transmembrane region. It also predicts the transmembrane helix and the inside and outside sequence in the transmembrane region. TMHMM server predicted the presence of three transmembrane region from residue 396-430 (TM helix1), residue 445-469 (TM helix2), and residue 474-496 (TM helix3) (Figure 3A) [37]. Programme COILS compares a protein sequence to the database of existing two standard Coiled-coils in order to derive a similarity score. The Coiled-coil conformation of a protein sequence is finally determined by comparison of this derived similarity score and distribution of scores in the globular Coiled-coil. Coiled-coil region prediction server COILS showed the presence of at least three intramolecular coiled-coil region in YspB (Figure 3B) [38]. Intramolecular coil-coiled regions play significant role in protein-protein interaction. YspB supposedly interacts with chaperone SycB and with other translocator proteins. YspB is an unstable protein and therefore, attempts for its recombinant expression failed because of its instability. This prompted us to check the presence of any disordered regions in YspB. Disordered region prediction servers predict the disordered probability within a sequence using their algorithms. Disorder prediction servers like PrDos and DisEMBL version 1.5 (with Loop or Coil, Remarks-465, Hot-Loops) predicted the presence of intrinsically disordered regions in the protein which provide an element of instability to YspB [39,40]. PrDos predicted disordered regions throughout the YspB protein sequence (Figure 4A). Loops or coil program predicted disordered regions in 1-8, 17-37, 53-69, 76-86, 92-107, 221-232, 310-323, 383-392, 533-543 and 598-610 residues. Hot-Loops program showed disordered regions in residues 1-12, 17-26, 49-64, 314-325, 361-372, 379-392, 566-579 and 661-676. On the other hand, Remark-465 program predicted disordered regions in 48-57, 72-106, 162-173, 364-390 residues (Figure 4B). Therefore, we could observe disordered regions spread throughout the protein sequence specifically clustered in the N-terminal of the protein and significantly less in the C-terminal region.

Homology Model of YspB depicts a Star shaped all helical structure interspersed by coiled regions

Foultier *et al.* 2003, pointed at the unstable and hydrophobic nature of YspB, which made raising antibody against YspB difficult

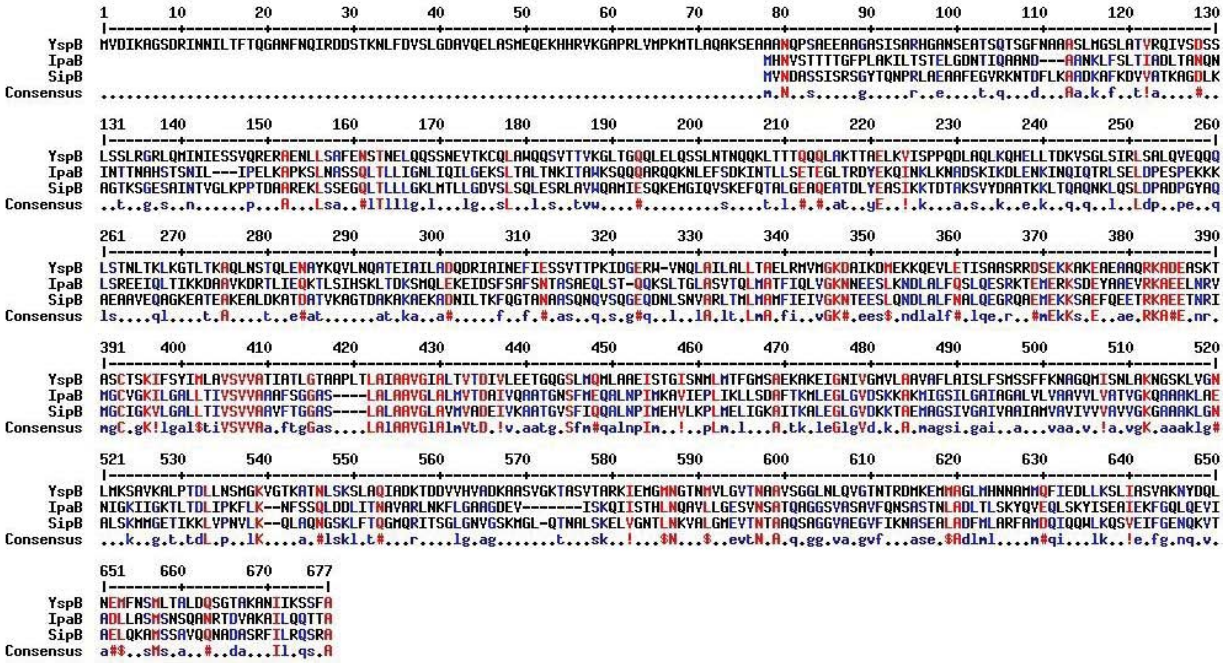

Figure 2: Multiple Sequence Alignment (MSA) showed significant homology between YspB and translocators from IpaB_SipB family: MSA profile of YspB, IpaB and SipB.

Table 2: Sequence identity, similarity and coverage between YspB and its homologs

	Sequence Identity	Sequence Similarity	Sequence Coverage
YspB & IpaB	12.85%	39.05%	85.67%
YspB & SipB	14.03%	40.38%	87.59%

[27]. In this study, we have marked the disordered regions in YspB. Also, SycB provides stability to YspB and in the absence of SycB, YspB degrades rapidly [27]. Under these circumstances recombinant expression of YspB to the level of three dimensional structure determination becomes very difficult. Therefore, a good homology model depicting scaffolds identical to the experimental structure would enable us to study protein-protein interaction and design drugs. I-TASSER is a leading server for prediction of three-dimensional protein structure based on homology modeling and threading approach. The templates for modeling were selected from PDB library using LOMETS. The best model was selected from the models generated using C-score, TM- Score and RMSD values [41].

Homology model of YspB was generated using I-TASSER server. C-score is a confidence score for estimation of the quality of the model predicted by I-TASSER [41]. The homology model of YspB had a C-score of -0.86, which indicates towards a good homology model. The estimated TM-score of 0.61 ± 0.14 lay the base for reliable prediction of the homology model of YspB. YspB model exhibited a star shaped structure. It contains 28 helices interspersed by loops (Figure 5A & 5B). The model could be divided into five distinct regions forming the five arms of the star. Region I consists of 3 helices; H1, H2 and H3. Region II consists of 9 helices; H4, H5, H6, H7, H13, H14, H15, H16 and H17. These helices together constitute the core region of the protein surrounded by the 5 helices of the Region III; H8, H9, H10, H11and H12 and 7 helices of Region V; H22, H23, H24, H25, H26, H27 and H28. Region IV consists of 4 helices; H18, H19, H20, H21. Out of 28 helices, helix H10 and H12 are the two longest helices, which might participate in intramolecular interaction resulting in formation of YspB dimer and oligomer. All these helices are interspersed by loops. The longest loop is of 14 amino acid residues and exists between helices H8 and H9 (Figure 5C). The YspB model is an elongated one showing a maximum length of 100.66 Å and 96.99 Å (Figure 5D). Therefore, the star shaped structure of YspB appears as a union of 5 different bundles of helices joined by looped regions. The three dimensional structures of major translocator proteins are rare and therefore, they could prove to be beneficial in deciphering translocator- chaperone interaction. Also, this three dimensional model of YspB could be used to study its interaction with other translocator proteins like YspC and YspD and its interaction with the host cell plasma membrane. This model of YspB could be potentially beneficial for drug designing to deregulate Ysa-Ysp T3SS.

SycB partially fits within the YspB structure by an interaction mediated by the two TPR regions forming the concave core of SycB

Dynamic Light Scattering profile of SycB in its dimeric form shows a Hydrodynamic Diameter of 6.448 nm, which corroborates the dimensions of the dimeric model of SycB, generated by molecular docking studies (Figure 6A) [46]. The molecular docking studies were performed by ZDock molecular docking server. Here, the three dimensional structures of both the proteins could be uploaded. Also, ZDock allows selection of residues involved in interaction between the two proteins. In a similar manner, amino acids not present in the interaction interface could be marked by the user. Therefore, supplementary experimental data could be fed into the server for generation of model for protein-protein interaction. The residues of SycB interacting with YspB mainly reside in the two TPR regions of SycB (Figure 6B & 6C). SycB binds to the pocket formed by Region II, III, and IV of YspB. Helices H8 of Region III, H18 and H19 of Region IV and the loop between helices H14 and H15 of Region II are the

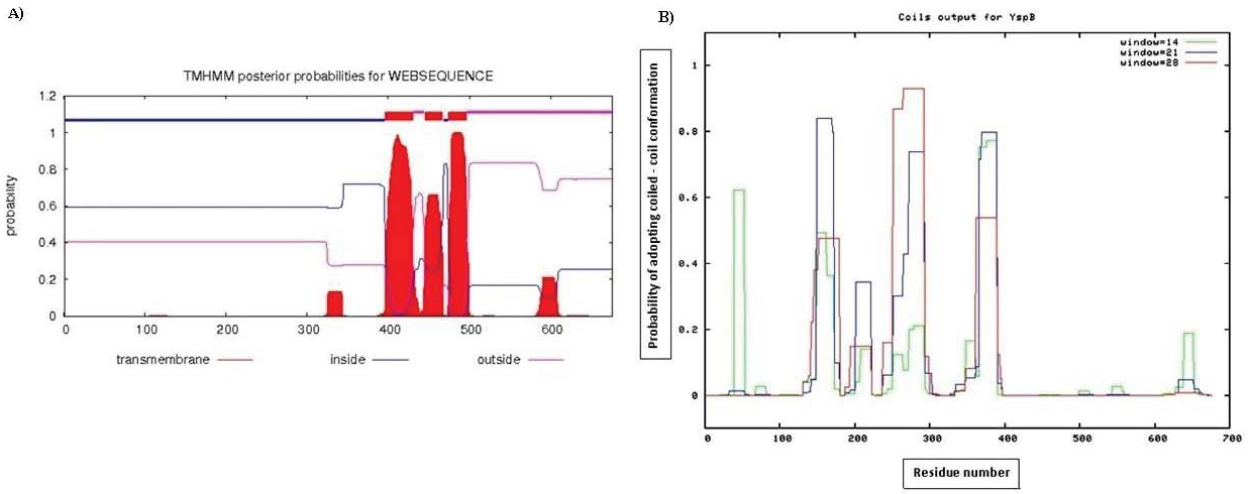

Figure 3: YspB possesses transmembrane and intramolecular coiled coil regions, characteristic of translocator proteins: A) Transmembrane regions in YspB predicted by TMHMM. B) Intramolecular coiled-coil regions present in YspB, predicted by COILS server.

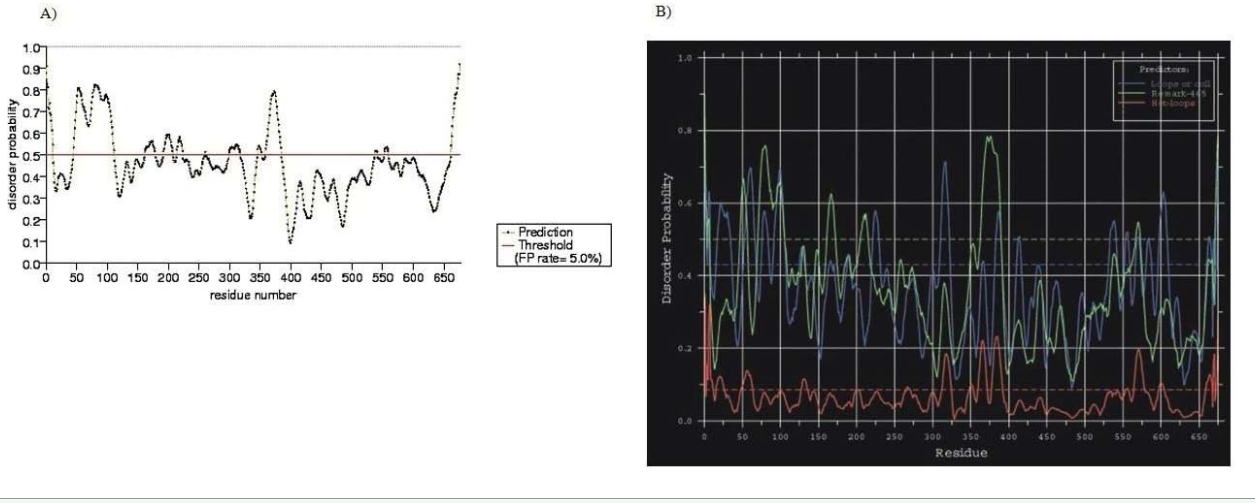

Figure 4: Intrinsically disordered regions exist throughout YspB resulting in the unstable nature of the protein: A) Disordered regions predicted by PrDos. B) Intrinsic disordered regions within YspB were determined by the programmes Loops or Coil, Remark- 465 and Hot- Loops of DisEMBL 1.5 server.

helices mainly interacting with SycB. Helices H18 and H19 of YspB interact with helices H3 and H4 of TPR1 of SycB. The loop between helices H14 and H15 of YspB participate in the interaction with helix H5 of TPR2 of SycB. Helix H8 of Region III of YspB interacts with the loop region between the helices H6 and H7 of SycB. Thus, H7, H8 and H9 of SycB and all other helices of YspB might not involve in any kind of interaction (Figure 7A & 7B). The interaction between SycB and YspB is similar to the existing models of translocator-chaperone interaction. For instance, IpgC (a close homologue of SycB) interacts with IpaB-peptide by pockets P1, P2, and P3, which are mainly formed by the residues in the first two TPR regions of the IpgC molecule [47]. In Psc T3SS of *Pseudomonas aeruginosa*, TPRs of PcrH forms the concave cleft involved in interaction of PcrH with PopD and major translocator PopB [48]. The maximum length of YspB-SycB complex is 127.83 Å and 96.35 Å indicating that SycB does not completely fit into the YspB pocket, probably, because of the helices H13, H9 and H12 (Figure 7C).

The residues participating in the interaction between YspB and SycB are conserved in both the proteins. Polar residues of SycB like D109, Y79, Y47, Y49 and T42 are involved in interaction with YspB. These residues reside in the two conserved TPR regions (TPR1 and TPR2) of SycB. Similarly, polar residues like Q194, S491, S496 and K500 of YspB participate in the interaction with SycB (Figure 7D & 7E). These residues are located in helix H8, H18 and H19 of YspB. As polar and charged amino acids are mainly involved in the YspB-SycB interaction, therefore, the interaction could be ionic or polar in nature. In some of the cases, these residues might form ion pairs or salt bridges among themselves and provide stability to both the proteins in the complex from. In that case, increase in the salt concentration in the environment will be responsible for the dissociation of SycB from YspB pocket. Therefore, the domains of SycB interacting with the major translocator YspB and minor translocator YspC are same and confined mainly to the first two TPR regions. Hence, SycB cannot simultaneously bind to YspB and YspC. The dimerization domain of SycB is different and restricted to the N-terminal helix [33,49]. But the interaction with bulky translocator proteins like YspB or YspC results in dissociation of the dimeric physiological state of SycB [46]. Understanding the structural basis of translocator-chaperone

interaction would enable us to understand better the mechanism of regulation of T3SS. Further, if the nature of interaction and specific residues involved in interaction could be pinpointed, the affinity of the interaction could be regulated for therapeutic purposes.

ConSurf depicted that the residues of YspB involved in interaction with SycB are evolutionarily conserved

ConSurf is a web based tool for the prediction of structurally and functionally conserved regions in proteins [43]. ConSurf performs a BLAST search, generates a MSA of the input protein and its homologs and further generates a phylogenetic tree. Therefore, the evolutionarily conserved regions within the protein could be predicted using this web based tool. In YspB, helix H18 and loop between helices H14 and H15 involved in YspB-SycB interaction show high degree of evolutionary conservation. Also, helix H19 and helix H8 involved in interaction of YspB with its chaperone show partial conservation. As these helices form the interface of protein-protein interaction, the conserved residues within them are functionally significant. However, conserved residues which are structurally important could be observed in helix H1, H2 and H3 and in looped region between H18 and H19 (Figure 8A & 8B). Besides this, structurally and functionally conserved residues are scattered throughout the YspB structure. Hence, major translocator YspB of Ysa-Ysp T3SS shows significant evolutionary conservation. Its lineage could be predicted from the phylogenetic tree generated by ConSurf. This is in contrast to YspC (minor translocator of Ysa-Ysp T3SS), which is a unique protein [49]. These results could help to decipher the evolutionary lineage of comparatively less studied Ysa-Ysp T3SS.

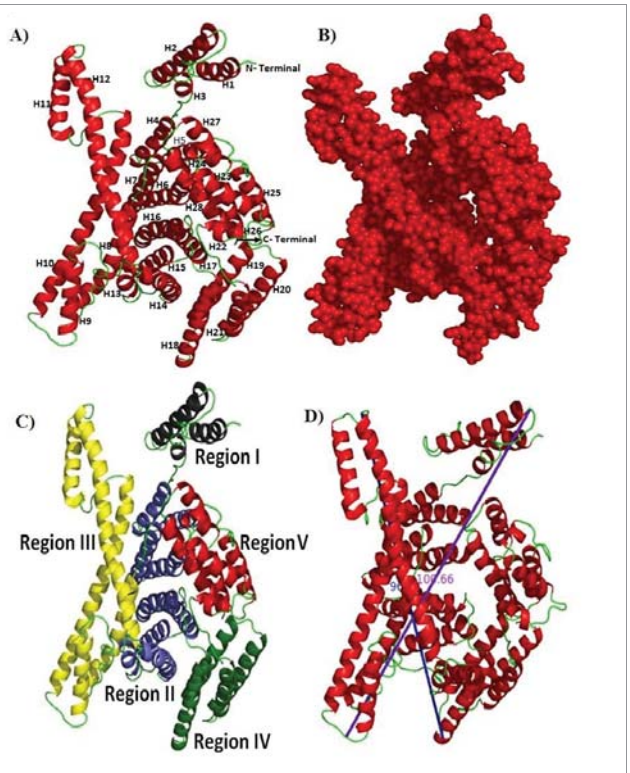

Figure 5: Homology model of YspB exhibits a star shaped all helical structure interspersed by coiled regions: A) Cartoon representation of YspB structure. The helices were shown in red and the coiled regions were shown in green. B) The atoms of the YspB mdel were represented as sphere. C) Cartoon representation of YspB model divided into five distinct regions where Region I was shown in grey 40 and Region II, III, IV and V were shown in slate, yellow, forest and red, respectively. D) The dimensions of YspB structure were marked in the cartoon representation of the homology model of YspB.

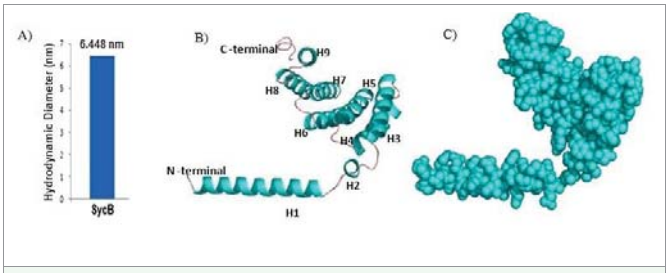

Figure 6: Dynamic Light Scattering (DLS) corroborates the dimensions of In Silico model of SycB: A) DLS profile showing the hydrodynamic diameter of SycB dimer. B) Cartoon representation of SycB structure. The helices were shown in cyan and the coiled regions were shown in magentas. C) The atoms of the SycB model were represented as sphere.

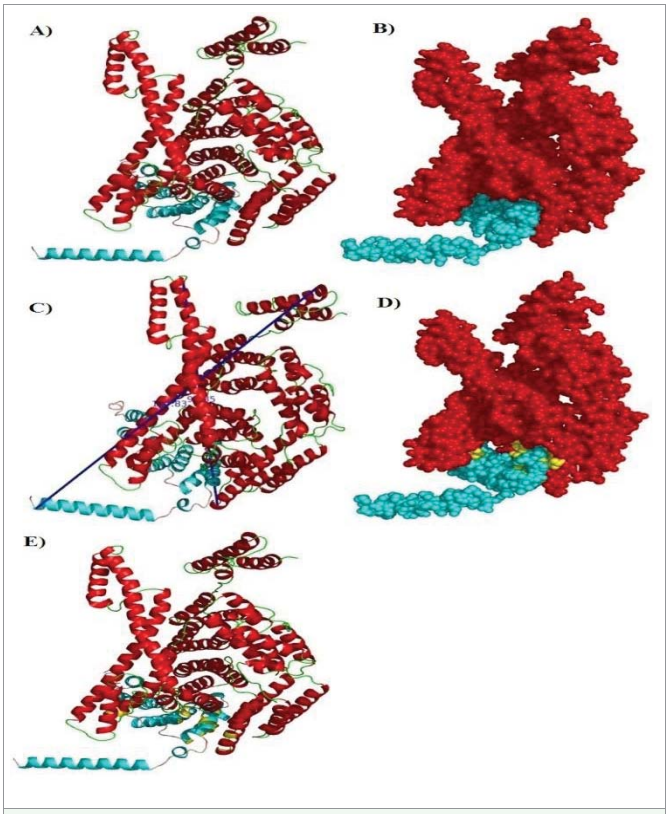

Figure 7: SycB partially fits within the YspB structure by an interaction mediated by the two TPR regions forming the concave core of SycB: A) Cartoon representation of YspB-SycB complex. The helices of YspB were shown in red and coiled regions were shown in green. The helices of SycB were shown in cyan and coiled regions were shown in magentas. B) The atoms of the YspB-SycB interaction model were represented as sphere. The atoms of YspB were shown in red and atoms of SycB were shown in cyan. C) The dimensions of YspB-SycB complex were marked in the cartoon representation of YspB-SycB interaction model. D) The atoms of YspB-SycB model were shown as spheres and the interacting residues were represented in yellow. E) Residues involved in YspB-SycB interaction were marked in yellow in the cartoon representation of YspB-SycB model

CONCLUSION

In Silico approach of structural analysis enabled us to decipher some characteristic features of YspB protein. High alpha helical content, presence of transmembrane helices, intrinsically disordered regions and intramolecular coiled-coil are the characteristic features

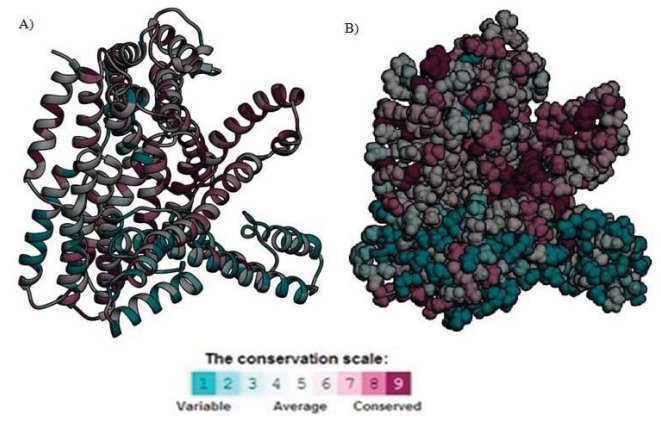

Figure 8: ConSurf analysis depicted that residues of YspB involved in interaction with SycB are highly conserved along with some structurally conserved helices: A) Cartoon representation of ConSurf generated model of YspB along with the colour graded conservation code. B) The atoms of YspB model generated by ConSurf were represented as spheres.

of major translocator proteins. It also showed significant homology to the proteins of IpaB_SipB family. Therefore, unlike minor translocator YspC of Ysa-Ysp T3SS, YspB is not a unique protein and it could be easily categorized as translocon forming protein. SycB interacted with YspB using its two TPR regions. The amino acid residues of YspB interacting with SycB are mostly evolutionarily conserved. Also, the nature of residues responsible for YspB-SycB interaction depicted mainly ionic or polar interaction between these proteins. However, the interacting regions of SycB were same for both YspB and YspC and different from the dimerization domain of SycB. So, simultaneously SycB cannot bind to YspB and YspC. Also, interaction with either of the translocator results in dissociation of dimeric state of SycB due to steric hindrances. Further, these interacting interfaces are potential drug targets to disrupt translocator-chaperone interaction, which could result in deregulation of Ysa-Ysp T3SS and attenuate the virulence of *Yersinia enterocolitica* Biovar 1B.

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