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DETERMINING THE CRYSTAL STRUCTURE OF SSEB, A PRODUCT OF THE SALMONELLA PATHOGENICITY ISLAND II TYPE III SECRETION SYSTEM OF SALMONELLA TYPHIMURIUM

A Senior Honors Thesis

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ABSTRACT

Determining the crystal structure of SseB, a product of the *Salmonella* Pathogenicity Island II Type III Secretion System of *Salmonella typhimurium*

(April 2007)

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Salmonella typhimurium is a bacterium that causes many food-borne illnesses such as gastrointestinal infections, diarrhea, and abdominal cramps. It affects 700,000 to 3.8 million people each year. The SseB protein, a part of the *Salmonella* Pathogenicity Island II (SPI2), plays a critical role in the pathogenesis of gastrointestinal infections. It is part of the Type III Secretion System (TTSS) that is involved in translocating proteins from the bacteria to the host gastro intestinal-epithelial cells. The aim of the research project is to clone, purify the SseB protein from *Salmonella typhimuriu*m and obtain a

diffracting-quality crystal that will give high resolution data so that the structure of the protein can be determined using x-ray diffraction patterns.

The SseB gene was amplified and cloned into pET30b vector and transformed into *E. coli* novablue cells. The SseB protein is then expressed into *E. coli* BL21 cells and purified using various chromatographic methods. Purified protein was used for crystallization and diffracting quality crystals were obtained using grid screening method. SseB protein crystallized in P-orthorhombic space group (P 21 21 21) and diffracted to 3.8Å. Further optimization is underway to get a good diffracting quality crystal.

It is important to determine the crystal structure of SseB since this will reveal its role in the interaction with other translocation complex such as (SseC and SseD). Based on the structural information, potential drug targets can be designed for translocon complex, which can further prevent diseases caused by the bacterium *Salmonella* and other closely related bacteria.

DEDICATION

I would like to dedicate my work to my parents, Narendra and Padma Patel, my sister, Neha Patel, and my brother, Neel Patel.

ACKNOWLEDGEMENT

I would like to acknowledge Dr. Kuppan Gokulan, Dr. James Sacchettini, and Varun Chowdhary for their assistance and guidance with this research project.

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I. INTRODUCTION[1]

Salmonella typhimurium is a bacterium that causes many food-borne illnesses and gastrointestinal infections such as diarrhea and abdominal pain. This bacterium affects thousands of people every year, and it also causes gastro-intestinal diseases in many other animal hosts. *Salmonella* enter the intestine and survive in the acidic gastric barrier. It can infect the mucous barrier lining of the small and large intestine. Then the bacteria produce toxins which invokes an inflammatory response which causes the disease.

Secretion is a system that a cell uses to releases chemicals and molecules. Eukaryotic cells have various highly evolved secretion systems. The Type I secretion system involves an ATP binding cassette transporters while the Type II and the Type V secretion systems depend on the Sec system for the protein to cross the inner and the outer membrane (24, 25, 26). The Type III Secretion system is homologous to a bacterial flagellar basal body and is also involved in translocating proteins across the bacterial inner and outer membrane as well as the host membrane (7). The Type IV secretion system is homologous to the conjugation machinery of bacteria and can transport both

[1] This thesis follows the style and format of *Journal of Biological Chemistry.*

DNA and protein (27). The Type III secretion system is secretion system used by *Salmonella* Pathogenicity Islands.

The Type III Secretion System is a highly specialized bacterial protein secretary pathway and plays an essential role in the pathogenesis of many Gram negative pathogens including *Shigella, Salmonella, Bordetlla, Psueudomonas* and pathogenic *E. coli.* Recent studies have gained our knowledge on the structure and functions of many type III translocated effectors. However, the detailed mechanism of secretion, translocation and the detailed structural characteristics of secretion apparatus still poorly understood. The type III secretion complex consists of more than twenty proteins and are highly conserved many bacteria which have TTSS (7).

There are two clusters of the genes of *Salmonella* that play a key role in the pathogenesis and commonly known as *Salmonella* Pathogenicity Islands I & II (SPI1 & SPI2, respectively). The function of the genes within SPI1 is to allow the bacteria to invade and penetrate the gastrointestinal epithelial cells of the host. The genes within SPI2 encode many structural genes including the Type III secretion System (TTSS). This secretion system is used to transport proteins by forming a tunnel through which the

virulent proteins can be transported from the bacterial cytoplasm to the host cells. The effector proteins are the virulent proteins that affect the host cells, and the translocon are the proteins that enable the transfer of effector proteins (9).

There are many proteins involved in transferring the virulent factors; however, the SseB protein plays a critical role in forming the translocon along with other translocon proteins. SseB is a translocon which translocates effector proteins into host cells. The primary biological function of TTSS is not the secretion of proteins, but the translocation of effector proteins over a third membrane such as the membrane of the eukaryotic host cell (7).

Type III Secretion Systems are desirable targets for the development of novel-anti-virulence drugs as their inactivation would lead to pathogen attenuation or avirulence, followed by clearance of the pathogen by immune system. The purpose of this project was to purify and crystallize the SseB protein so that the structure can be determined. The three-dimensional structure will also provide its role in TTSS and it can be used to design drugs that can act as inhibitors which will prevent the formation of the translocon.

Thus, the effector proteins will not be able to translocate from the bacterial cytoplasm to the host cells preventing many diseases caused by this bacterium.

II. LITERATURE REVIEW

Salmonella leads as one of the bacteria that cause food-borne illness globally. It survives inside the host cells and proliferates in vacuoles (9). *Salmonella* is a gram negative bacteria, which like other gram negative bacteria, have evolved a system that solves the problem of translocating protein across two membranes of cell envelope by using the Type III Secretion System (TTSS). The main biological purpose of this system is not to secrete proteins to the media, but to translocate vectorially the effector proteins over a third membrane system, that is the plasma membrane of the eukaryotic host. This translocation plays a critical role in the pathogenesis and is important in the relationship between the bacteria and the hosts (10).

The translocation of virulence proteins into cells of the infected host is crucial for the pathogenesis of infections by many gram-negative bacteria. Type III secretion system (TTSS) is involved in various different aspects of pathogenesis (11). There are many sets of proteins that are secreted by TTSS. One subset of substrate proteins act as

translocated effector proteins and another subset of substrate proteins are required for the translocator of effector proteins, and thus acts as a translocator (1, 12).

Genes within the *Salmonella* Pathogenicity Island I (SPI1) include large clusters of invasion genes. In addition, TTSS, several effector proteins and regulators of gene expression are encoded by SPI1. As in figure 1, one subset of the SPI1 effector proteins modifies GTPases of host cell that affects the arrangements of host cell actin cytoskeleton and leads to the formation of membrane ruffles (6).

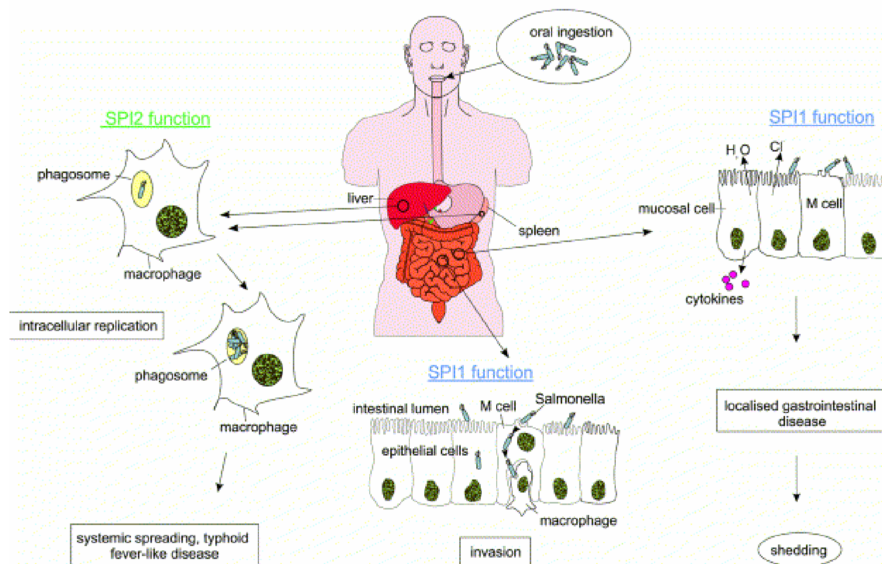

Figure 1: Functions of the genes on the *Salmonella* Pathogenicity Islands (7)
The SPI1 genes encode several proteins, which initiates the invasion in the host organism. Specifically it interacts with gastrointestinal epithelial cells and subsequently enters into the host cells. SPI2 genes encode for Type-III secretion apparatus, translocation complex and virulence factors.

Genes within the *Salmonella* Pathogenicity Island II (SPI2) also encode a second TTSS and expresses many of the structural genes. It encodes effectors that are activated after it is translocated into host cells. Three of the SPI2 encoded proteins, SseB, SseC, and SseD function as a translocon for effector proteins that are translocated into the host cells (6).

The TTSS apparatus is composed of about twenty to twenty-five different proteins. About half of these are conserved in most TTSS systems and are similar in sequence to the proteins of the basal body of the bacterial flagellum (11, 13, 14). This may indicate a shared evolutionary history; TTSS may have arose early in evolution through duplication of certain flagellar genes or it may be as ancient as the flagellar apparatus and they share a common ancestor (15). Protein secretion is common in both the flagellar and type III systems. The flagellum functions as a rotary motor powered by transmembrane ionic potentials, and its assembly requires secretion of flagellar subunits in a process powered by ATPase (16, 17, 18). TTSS also depends on ATPase as well as transmembrane ionic potential for protein transport (2).

The most prominent feature of TTSS is a needle-like structure and pili, which appears to be more flexible compared to the rigid needle (2). They may suggest the function of protein translocation between the bacterium and the host cell. As in figure 2, the needle-like structures are straight, rigid, and hollow. They look like a tunnel through which proteins are translocated into host cells and are much shorter than flagella, 80 nm in *Salmonella* (19). The needles are fairly uniformly dispersed over the surface of the bacterial cell (20). Folded protein domains often have diameters of 20 Å to 30 Å, which suggest that if effector proteins were transported through the interior of the needle, they would need to be partially or fully unfolded. A variation of the needle in pathogenic Escherichia coli *(E. coli)* has long filaments that appears to be attached to the end of the needle distal to the bacterial cell and is composed of the protein EspA (21).

The type III Secretion System is a very complex secretory system and it is found only in gram-negative bacteria. TTSS plays a major role in antiphagocytic and cytotoxic effects on host cells, invasion of host cells, intracellular pathogenesis, and the establishment of symbiotic relationships (6).

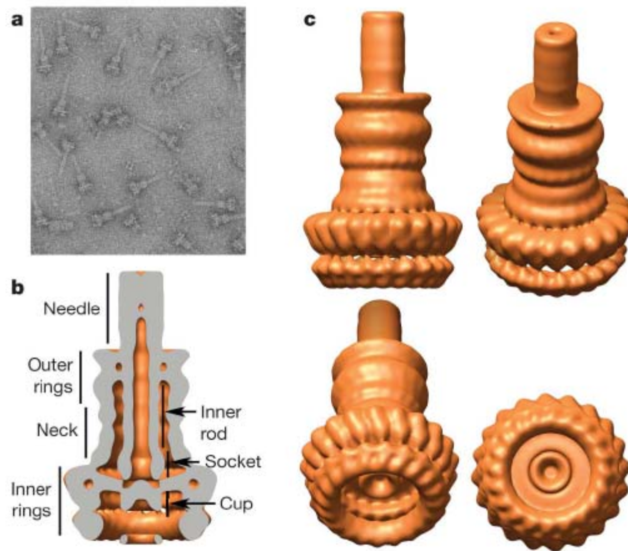

Figure 2: The needle complex of *Salmonella typhimurium* (8)

a) Electron micrograph of an isolated needle complex.

b) Cross-section of the structure of the needle complex indicating the location of the substructures.

SseB is part of a family of proteins which are found only in enterobacteria. It is known that SseB enhances serine-sensitivity in *E. coli* and is part of the *Salmonella* Pathogenicity Island II (SPI2) translocon. The SPI2 is composed of the effector region that encodes several secretion targets. As seen in figure 3, SseB has been targeted in the secretion system because of its similarity with the EspA, a protein secreted by TTSS secretion of enteropathogenic *E. coli*. As in figure 3, SseB is a secreted protein that associates with the bacterial cell surface and may be necessary for the delivery of SPI2

effector proteins to the eukaryotic cell cytosol (3). The structure of the SseB protein can provide its role in the translocation as well as interaction with other proteins.

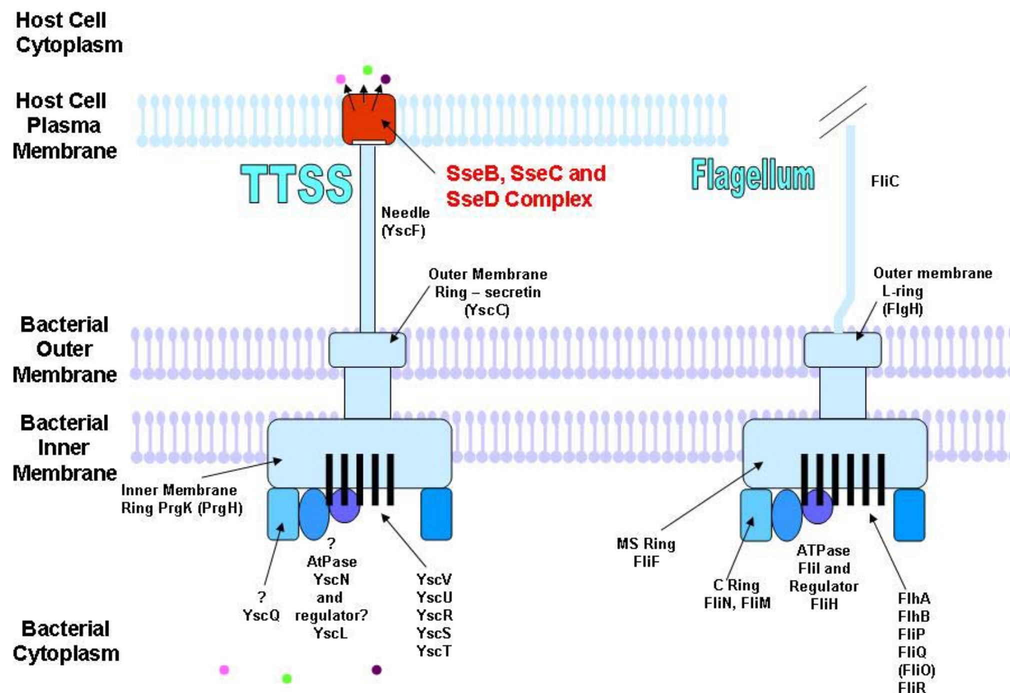

Figure 3: Schematic of the Type III Secretion System and a bacterial flagellum (2)
The translocon is a complex of SseB, SseC, and SseD and is involved in translocating the effector proteins (colored circles). The diagram also shows the homology between the type III secretion system and the bacterial flagellum.

The primary amino acid sequence alignment reveals that the SseB protein has twenty-three percent sequence identity and a forty-five percent sequence homology with the EspA protein of *E. coli*. The alignment of the SseB to the subunit of the translocon of

EspA is shown in figure 4. The C-terminal of SseB and EspA are sixty percent similar and the predicted SseB coiled-coil aligns with the EspA coiled-coil domain (23).

```
SseB  19  S F G V S N A D T G S Q D D L - S Q Q N P F A E G Y G V L L I L L  50
EspA  21  A Y D L G S M S K D D V I D L F N K L G V F Q A A I L M F A Y M Y  53

SseB  51  M V I Q A I A N N K F I E V Q K N A E R A R N T Q E K S N E M D E  83
EspA  54  Q A Q S D L S I A K F A D M N E A S K E S T T A Q K M A N L V D A  86

SseB  84  V I A K A A K G D A K - T K E E V P E D V I K Y M R D N G I L I D  115
EspA  87  K I A D V Q S S S D K N A K A Q L P D E V I S Y I N   P R N D I T  119

SseB 116  G M T I D D Y M A K Y G D H G K L D K G G L Q A I K A A L D N D A  148
EspA 120  I S G I D N I - - - - - - - N A Q L G A G D L Q T V K A A I S A K A  146

SseB 149  N R N T D L M S Q G Q I T I Q K M S Q E L N A V L T Q L T G L I S  181
EspA 147  N N L T T T V N N S Q L E I Q Q M S N T L N L L T S A R S D M Q S  179

SseB 182  K W G E I S S M I A Q K T Y S                                      196
EspA 180  L Q Y R T I S G I S L G K _ _                                      192
```

Figure 4: Sequence Alignment of SseB of *Salmonella* to the EspA of *E. coli*

This figure shows the homology between the SseB protein of *Salmonella* and the EspA protein of *E. coli*. The residues in red are identical in both while the residues in blue are similar in both.

The protein SseB in *Salmonella typhimurium* is related to the protein EspA in *E. coli*; however the existence of SseB filaments has not been demonstrated. The central channel in the filament may be necessary for the delivery of protein, suggesting that the filament serves as a continuation of the needle for protein transport (22, 23). Surface appendages much larger than needles and filaments have also been observed in *Salmonella typhimurium*. The relationship of these surface appendages to TTSS needle-like structures is unclear (2).

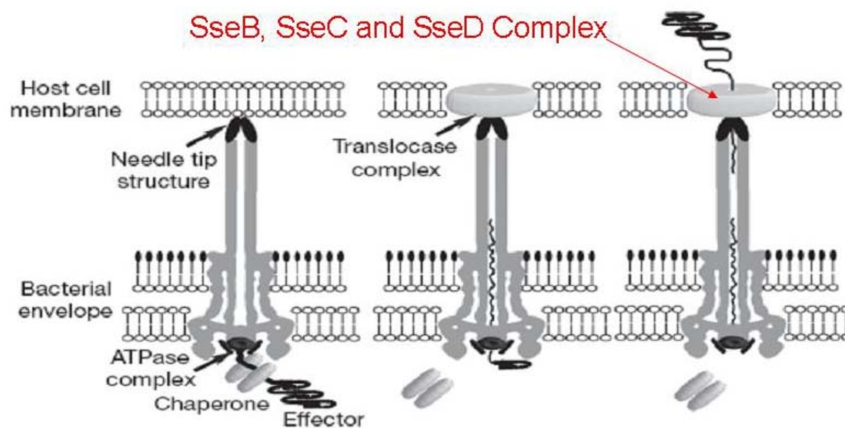

Figure 5: Model for delivery of proteins by Type III Secretion System (8)
The secretion system recognizes the effector-chaperone complex. The ATPases 'strips' the chaperone from the complex and the effector unfolds and 'threads' through the tunnel of the needle complex. The translocon made of SseB, SseC and SseD secreted by the Type III secretion system then translocates these effector proteins through the host cell membrane. The effector proteins refold within the host cell and carry out their function.

SseB plays an important role in secretion. SseB-containing structures are not distributed equally on the bacterial surface but are concentrated on one pole of the cell (6). *In-vitro* analysis of SseB, SseC, and SseD indicates that all three proteins are found to be loosely attached to the bacterial surface. Furthermore, the secretion of the SseB protein is independent compared to that of SseC, and SseD. However, the secretion of SseC and SseD occurred only in the presence of SseB. It is also believed that SseA acts as a chaperone for SseB and SseD (6).

Sensitivity to mechanical shearing indicates that after secretion by TTSS, SseB as well as SseC and SseD are present mainly on the surface of bacterial cells, suggesting their association in surface-located macromolecular structure. It may also be that the substrate proteins secreted into the growth medium associate with the cell surface due to unspecific interaction such as polar interactions between proteins and charged residues in the outer membrane. Previous experiments have shown that after secretion of SseC and SseD are located in cell-associated fraction if SseB is present, but they appear in the culture supernatant if SseB is absent (6). Thus, the structure of SseB could provide information about its role in the translocation of SPI2 effector proteins (1).

III. EXPERIMENTAL PROCEDURES

Cloning the SseB gene

Salmonella SseB gene contains 783 nucleotides and base pairs 1 to 600 were amplified by PCR from *Salmonella* genomic DNA, as the template using forward and reverse primers. The amplified DNA fragment was digested by Nde-1 and Hind-III enzymes (New England Biolab, Ipswich, MA). The digested DNA fragment was ligated into pET30b vector (Novagen, Madison WI) containing a C-terminal His_6-tag. The ligated plasmids were transformed into novablue cells (Novagen, Madison WI). Ten colonies

were taken from the transformed plate and grown in 5 ml LB media for ten hours at 37 °C. The cells were then centrifuged and the plasmid was extracted using the miniprep kit (Qiagen, Valencia, CA). The plasmid was then digested with the Hind-III and Nde-I restriction enzymes to screen for positive colonies as indicated by a band at the molecular weight of the plasmid and a band at the size of the SseB gene. The SseB-pET30b was introduced and transformed into *E. coli* BL21 cells (Novagen, Madison WI) for expression and 5 mL cultures were grown in LB media.

Media Preparation

The positive colonies were then transformed into LB media. The LB media was prepared by adding 20 grams of bactotryptone, 10 grams of yeast extract, and 20 grams of NaCl to 2 liters of ddH_2O.

Protein Expression

The BL21 (Invitrogen, Carlsbad, CA) cells containing the SseB gene were grown in LB media with the kanamycin antibiotic (Research Products International Corp., Prospect, IL). The cells were first grown and expressed at 16 °C, 20 °C, 25 °C, 30 °C, and 37 °C. The cells grown at 37 °C had the best expression. Large scale cultures were grown at 37 °C until the optical density (O.D.) reached 0.6. Then, the cells were induced with IPTG

(Research Products International Corp., Prospect, IL) for six hours to 1 mM final concentration. The cells were then centrifuged at 4000 rpm for 30 minutes and cells were stored at – 20°C until use. The cells were thawed and for every five grams of cell resolubilized with three volumes of Buffer A containing 20 mM Tris, 500 mM NaCl, and 50 mM imidazole containing protease inhibitors benzamidine (1mM), PMSF (1mM) DNase and $MgCl_2$ and kept in ice for thirty minutes. The cells were then lysed using a French Press three times at 1300 PSI and sonicated twice. The total cell lysate was centrifuged at 15000 K for one hour and the supernatant was collected and filtered using 0.45 micron filter.

Protein Purification

Nickel Exchange Chromatography is a purification technique where the protein's affinity to nickel (due to the histag) is used. When the supernatant is loaded onto the nickel column, the SseB protein with the histag (negative charge at used pH) will bind to nickel column (positive charge) and everything else will flow through. Then a gradient of increasing concentration of imidazole (highly negative charge) is passed through the column. Since imidazole has a higher affinity towards nickel, the bound SseB protein can be displaced from the column and be eluted.

To purify the SseB protein, the clear supernatant was loaded onto a Hi-trap nickel chelating column (Amersham Biosciences, Pittsburgh, PA) at a flow rate of 5 mL/min. The column was then washed with 300 ml of Buffer A containing 20 mM Tris, 500 mM NaCl, 50 mM Imidazole until the optical density reached zero. The His-tagged SseB was eluted using buffer containing 20 mM Tris, 500 mM NaCl, 500 mM Imidazole.

Ion-Exchange Chromatography is another purification technique that is based on the charge properties of the protein. An anion exchange column Q-sepharose was used to purify the SseB protein. The active group of Q-sepharose (Amersham Biosciences, Pittsburgh, PA) matrix is $CH_2N^+(CH_3)_3$ which have a positive charge, and negatively charged molecules on the protein are exchanged for one another. When the NaCl salt concentration is increased, the negatively charged chlorine atoms bind competitively to the positively charged Q-sepharose matrix, and the SseB protein can be eluted. The column was loaded with the protein and first washed with buffer A containing 20 mM Tris. A gradient of buffer A and buffer B containing 20 mM Tris, 1mM 2-ME, 1mM EDTA and 1 M NaCl was used to elute the protein. After purification to near homogeneity by size exclusion chromatography on S-Superdex-200 column, SseB was

dialyzed against 20 mM Tris and concentrated using centricon prep 10K (Amicon-Millipore, Billerica, MA) to a final concentration of 10 mg/mL and stored at -80 °C.

Preparation of Selenomethionine Protein

Selenium is a heavy metal that can be used to get the phase information of protein crystal. Selenomethionine (Se-Met) will be incorporated into proteins instead of methionine and multiple anomalous data will be collected from Se-Met crystal. The Se-Met protein was expressed in M9 media containing 24.96 grams of Na_2HPO_4, 12 grams of KH_2PO_4, 2 grams of NaCl, and 4 grams of NH_2Cl adjusted with ddH_2O to a final volume of 100 mL. 200 mL of 5X M9 media is added to 800 mL of autoclaved water. Then, 1 mL of 1 $MgSO_4$, 10 mL of 40% glucose, 100 μL of thiamine vitamin and 1 mL of 4.2 g/L of $Fe(II)SO_4$ and 1 mL of the kanamycin antibiotic are added. Then, all the L-amino acids except methionine, and Selenomethionine are added. To induce the cells, 1 mL of 1 M IPTG is also added. After four to six hours, the cells are harvested.

Protein Crystallization

In order to form a protein crystal, it has to form a lattice as in ice or salt crystals. Once the protein crystallizes, x-ray diffraction techniques are used to determine the structure

of the protein. Initially, the SseB protein was set up using various conditions such as Crystal Screen I&II, Wizard I&II, Index, Peg-Ion, and Membfac. A 10 mg/mL protein concentration was used. The protein crystallized at 18 °C by vapor diffusion when equilibrated against 0.1M HEPES pH 7.5, 10 % w/v Polyethylene Glycol 6000, and 5 % v/v MPD. SseB protein crystallized in P-orthorhombic space group with P 21 21 21 indicating the presence of the dimer in the asymmetric unit.

The crystal was optimized at this condition using 4 μL hanging drops. Three drops were set up for the condition: SseB protein concentration of 10mg/mL, SseB protein and 0.05% DDM, and SseB protein with concentration of10 mg/mL diluted into MES buffer. Then, 2 μL of protein and 2 μL of well solution were used to set up all the conditions.

Structure Determination

The crystal was mounted in cryo-loops were flash cooled in a liquid nitrogen mounted nitrogen. Paratone was used as a cryoprotectant. The native data of SseB were recorded at APS beamline 19ID using 3 X 3 segment APS-1 CCD detector. The diffracted data was reduced using DENZO. The crystal diffracts the x-rays and creates a diffraction pattern. This produces a pattern of intensity spots and the spacing in the lattice can be

calculated using Bragg's Law. This is then used to make an electron density map which provides the spatial location of the atoms in the lattice. The atomic model or the structure is then determined by fitting the molecule to the spatial orientation of the atoms. The SseB crystal diffracted at 8 Å and further optimization improved diffraction quality from 8 Å to 3.8 Å.

IV. RESULTS AND DISCUSSION

Cloning the SseB gene

The SseB gene was amplified using *Salmonella* genomic DNA from *Salmonella typhimurium*. The amplified PCR product was run in 1% DNA agarose gel and it clearly shows that amplified product migrated just above 0.5K marker and matches to 600 bp product. The DNA gel also shows that there is no other non specific band or primer dimer formation. The last two lanes in figure 6 shows that the amplified SseB PCR product and thick single bands at 600 bp molecular weight. SseB PCR product and pET30b plasmid was digested with Nde-1 and Hind-III at 37 °C overnight. The digested product was once again run in DNA agarose gel (data not shown).

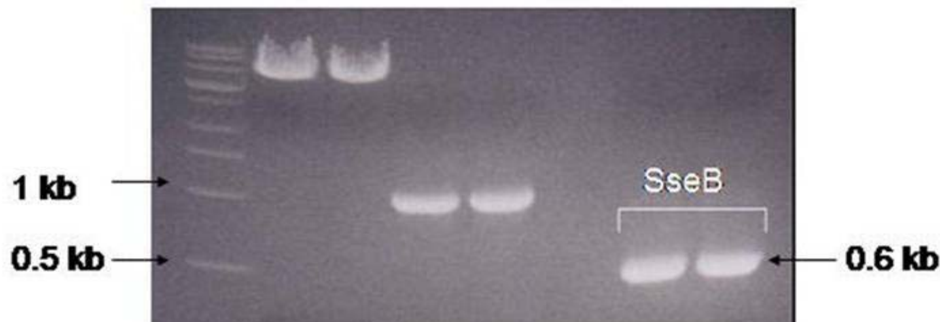

Figure 6: DNA agarose gel of the SseB

Lane 1 has a molecular weight marker (0.5K to 12K), lanes 2 and 3 have the pET30b vector, and lanes 7 and 8 have the SseB PCR amplified product.

The digested SseB PCR product and plasmid were cut from the DNA agarose gel and purified using Qiagen gel extraction kit. The PCR product was eluted using RNase free water. Digested SseB gene and pET30b plasmid were ligated by rapid ligation procedure. The ligation was done according to the manufacture's protocol (Roche, Indianapolis, IN). Different ratios of SseB and pET30b plasmid were used in different ratios (1:1, 2:1, 3:1 and 4:1) for ligation. The ligated sample was transformed into novablue cells and kept at 37 °C overnight. Colonies were observed on the transformed plates while no colonies were observed on the control plate indicating that the colonies on the transformed plates were positive colonies.

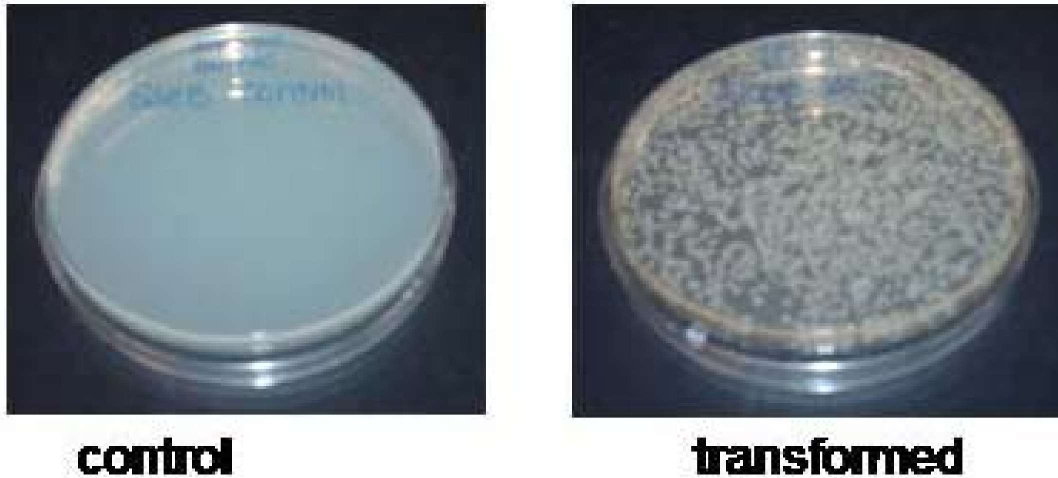

Figure 7: Positive Control

The control plate has no colonies while the transformed plate has positive colonies which have the SseB gene insert.

From the transformed plate, ten colonies were picked and grown in 5 ml LB media overnight at 37 °C. The plasmid was purified using Qiagen mini prep kit and eluted with RNase free water. Eluted plasmid (5µl) was used for double digestion in order to check the presence of SseB insert. After four hours of double digestion, these samples were run on DNA agarose gel. The result clearly shows that seven colonies were positive out of ten colonies.

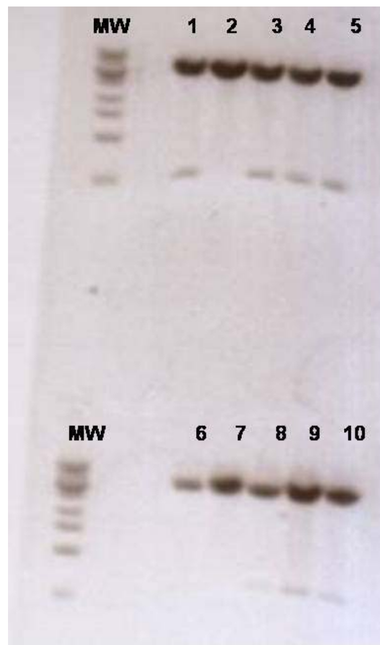

Figure 8: DNA agarose gel of positive colonies

The digested plasmid shows two band one at 5.2 kb and another at 600 bp. The lower band matches exact molecular weight of amplified SseB gene. The gel shows that 70% of the colonies were positive (lanes: 1, 3, 4, 5, 8, 9, 10).

Protein Expression and Purification:

To test the SseB protein expression, the positive plasmid was transformed into BL21, BL21Plys and Rosetta cells. The expression test was performed with various expression hosts and temperatures with and without IPTG. Cells were harvested and cell pellet was digested with lysis buffer. SDS gel was run to analyze the protein expression.

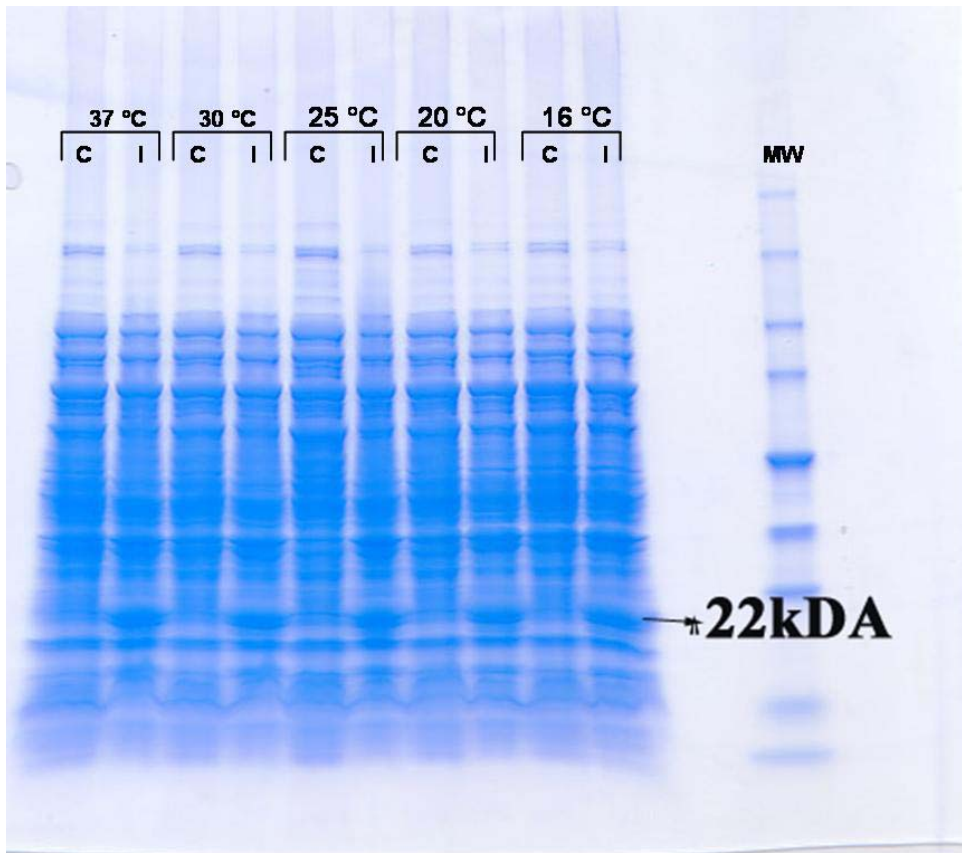

Figure 9: Protein Expression

The SDS page gel shows that the protein expresses at all the temperatures and the best expression is at 37 °C. The large thick band at 22 kDa indicates the SseB protein expressed when induced (I).

Large cultures of the gene expressed in Bl21 cells were grown in LB media and induced with IPTG. The cells were harvested, lysed, and centrifuged again. The supernatant containing the SseB protein was collected for further purification using Nickel Affinity Chromatography.

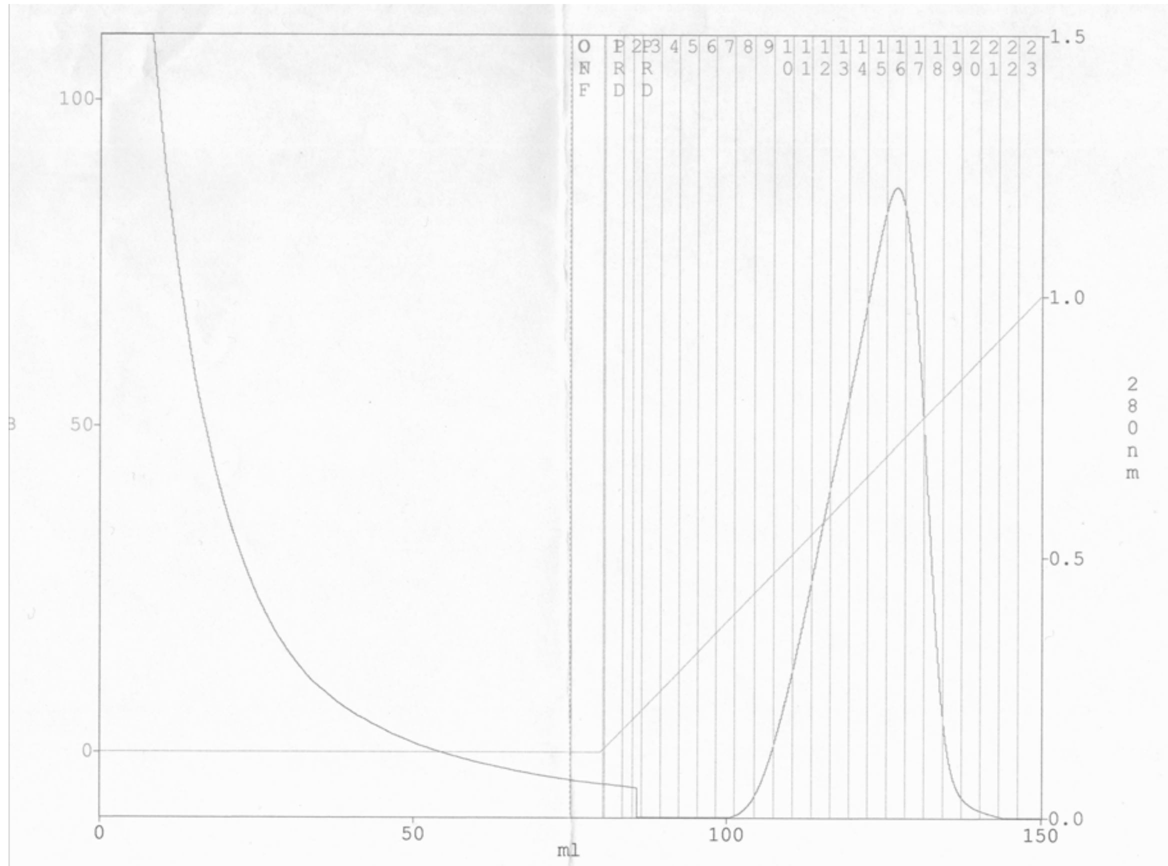

Figure 10: Chromatogram of Nickel Affinity Chromatography

The Chromatogram shows the elution profile of SseB protein. The bound protein was eluted from fraction 11 to fraction 18.

The fractions that contain the protein were then run on a SDS-PAGE gel to check the purity of the protein. The SseB protein is 22 kDa in size so a band at that molecular weight is expected in the gel. The SDS gel in figure 11 shows two bands: a major band at 22 kDa as well as a smaller band below it. This shows that the SseB protein has about eighty percent purity. Ion-Exchange column was used to remove the other smaller band.

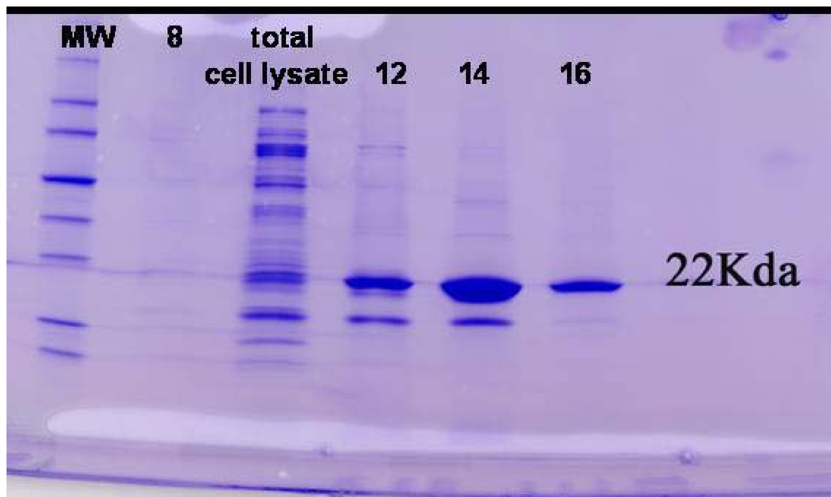

Figure 11: SDS gel of fractions after Nickel Affinity Chromatography

The gel shows thick band at 22 kDa indicating the SseB protein as well as another smaller band below it.

The Q-sepharose column was washed and the protein was loaded onto the column. The column was then washed again and the protein was eluted using a gradient with an increasing concentration of imidazole. The fractions were then analyzed by SDS gel. The gel shows a band at 22 kDa which is the correct molecular weight of SseB. The smaller band has been completely removed during ion exchange purification, and the SseB protein has ninety-five to ninety-eight percent purity.

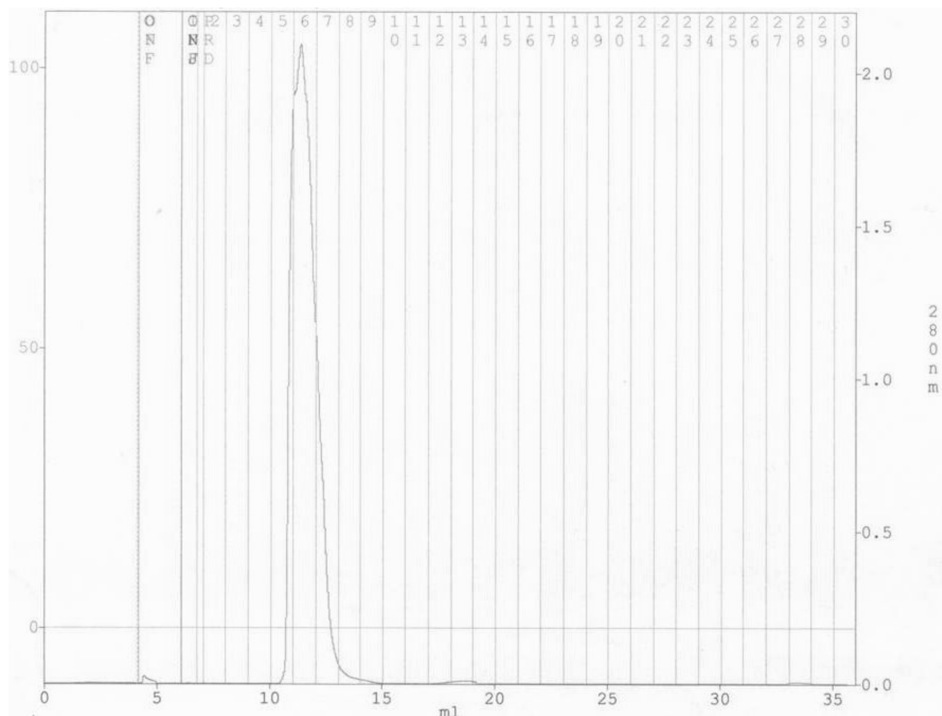

Figure 12: Chromatogram of Ion Exchange Chromatography

The Chromatogram shows the elution profile of SseB protein. The bound protein was eluted from fraction 5 to fraction 7.

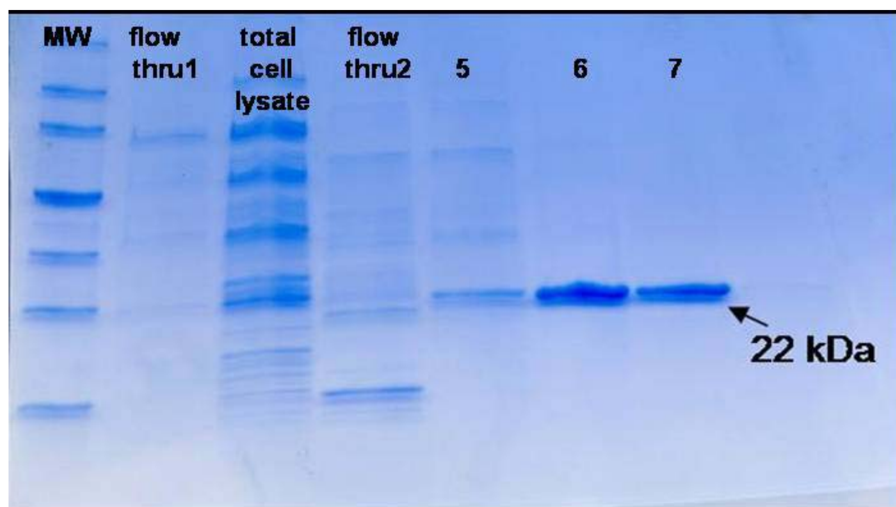

Figure 13: SDS Gel after Ion Exchange Chromatography

The SDS gel shows a thick band at 22 kDa which is the molecular weight of the SseB protein. The lower band has been removed and the protein is highly pure.

Protein Crystallization

The protein pooled after the ion-exchange column was then purified using size exclusion chromatography. The protein was then dialyzed and concentrated using a centricon prep 10 K. During the initial screening, plates were set up using Crystal Screen I & II, Wizard I & II, Peg Ion, Membfac, and Index. The crystal was obtained at the following condition: 0.1M HEPES pH 7.5, 10 % w/v Polyethylene Glycol 6000, and 5 % v/v MPD. The SseB protein crystallized in P-orthorhombic space group with P 21 21 21 indicating the presence of the dimer in the asymmetric unit.

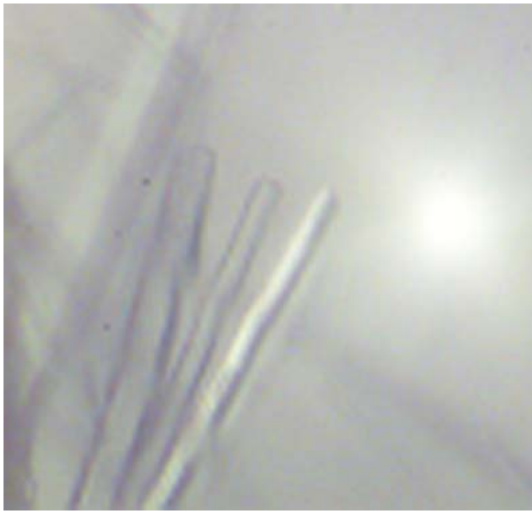

Figure 14: Photograph of the SseB protein crystal
This figure shows that the SseB crystal is rod shaped.

Structure Modeling and Structure Determination

The first crystal only diffracted at 8 Å and the recently obtained crystal diffracted at 3.8 Å. This resolution is not high enough to solve the structure of the protein. Currently, optimization of Se-Met crystal is under progress. The primary sequence alignment reveals homology between SseB and EspA. A partial SseB model was built using the structure of EspA.

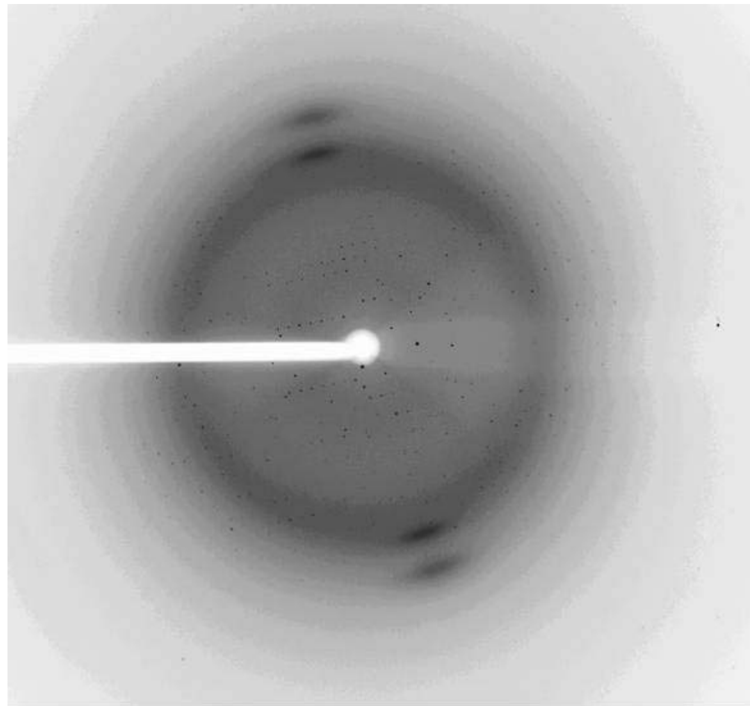

Figure 15: Diffraction pattern of the SseB protein
This is a picture of one of the frames collected of the x-ray diffraction pattern.

Atomic coordinates for the partial structure (3-58 and 158-196) of EspA is available form the Protein Data Bank at www.rcsb.org/pdb (PDB ID code 1XOU; (28).The atomic coordinates of 1XOU crystal structure was used as a starting model and pdb coordinated was read in the Xtalview (29) and mutations were made according to SseB sequence of *Salmonella typhimurium*. In particular, resides 3 to 58 of SseB has homology with 148 to 196 of ESPA while the residues 148 to 196 of EspA has homology with residues 158 to 196 of SseB. Hydrogen bonds were added to the modeled SseB structure. The modeled SseB structure was energy minimized in the Insight II suite, using Discovery (www.accelyrs.com).

The secondary structure of partially build SseB model has two α-helices. These two α-helixes are parallel together. The N-terminal domain is located near to the C-terminal domain. The short α-helix contains residues from 3 to 58 and larger helix contains residues from 158 to 196. Similar pattern of secondary structure was observed in many membrane proteins. Figure 18 shows surface area of the partially modeled SseB protein.

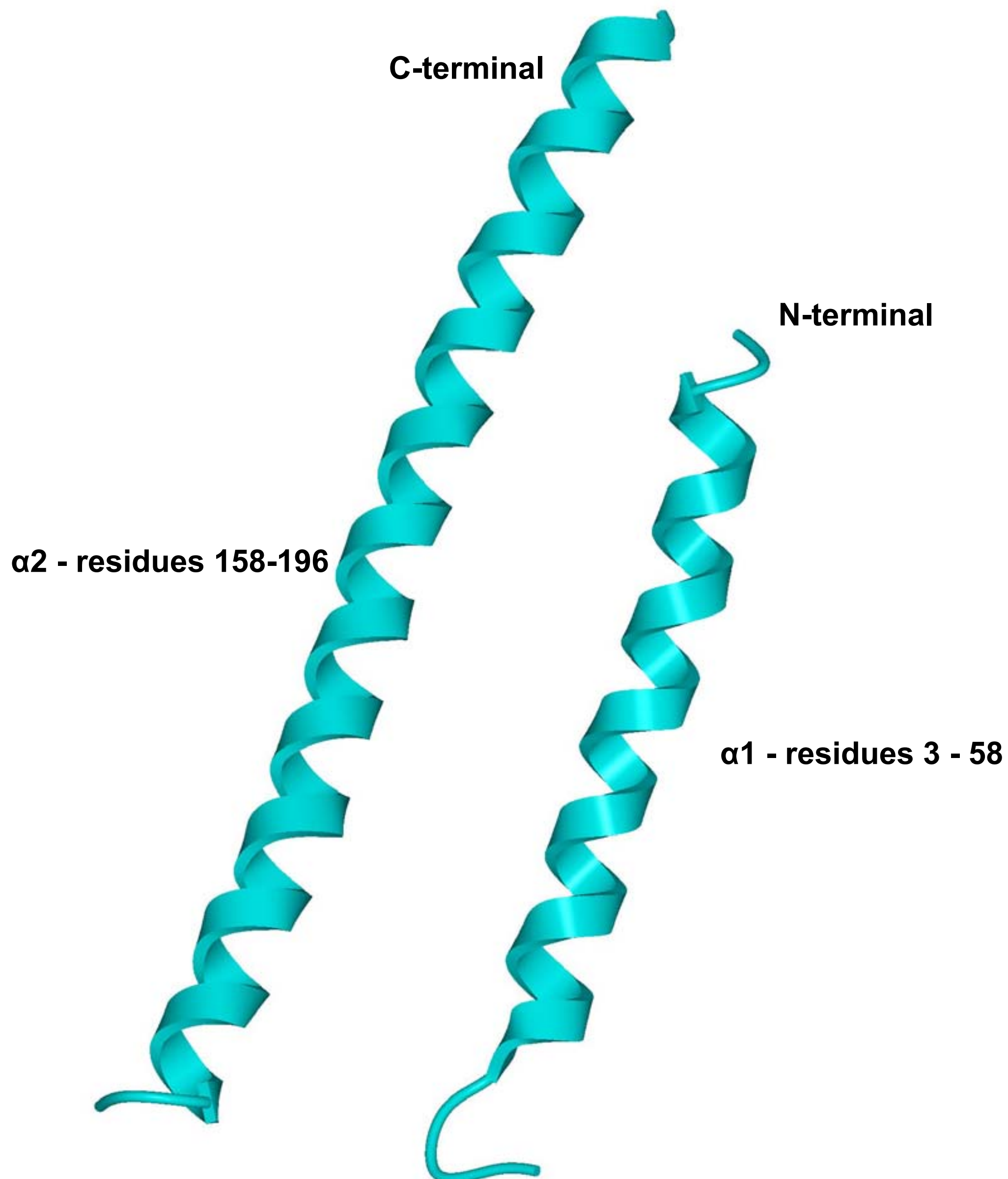

Figure 16: Overview of partial model of SseB protein of *Salmonella typhimurium*

The ribbon represents the secondary structure elements of SseB protein. Partial model of SseB contains two α-helices, the short helix contains residues from 3-58 and long helix contains 158-196. This SseB model was build using 1XOU PDB coordinates (28).

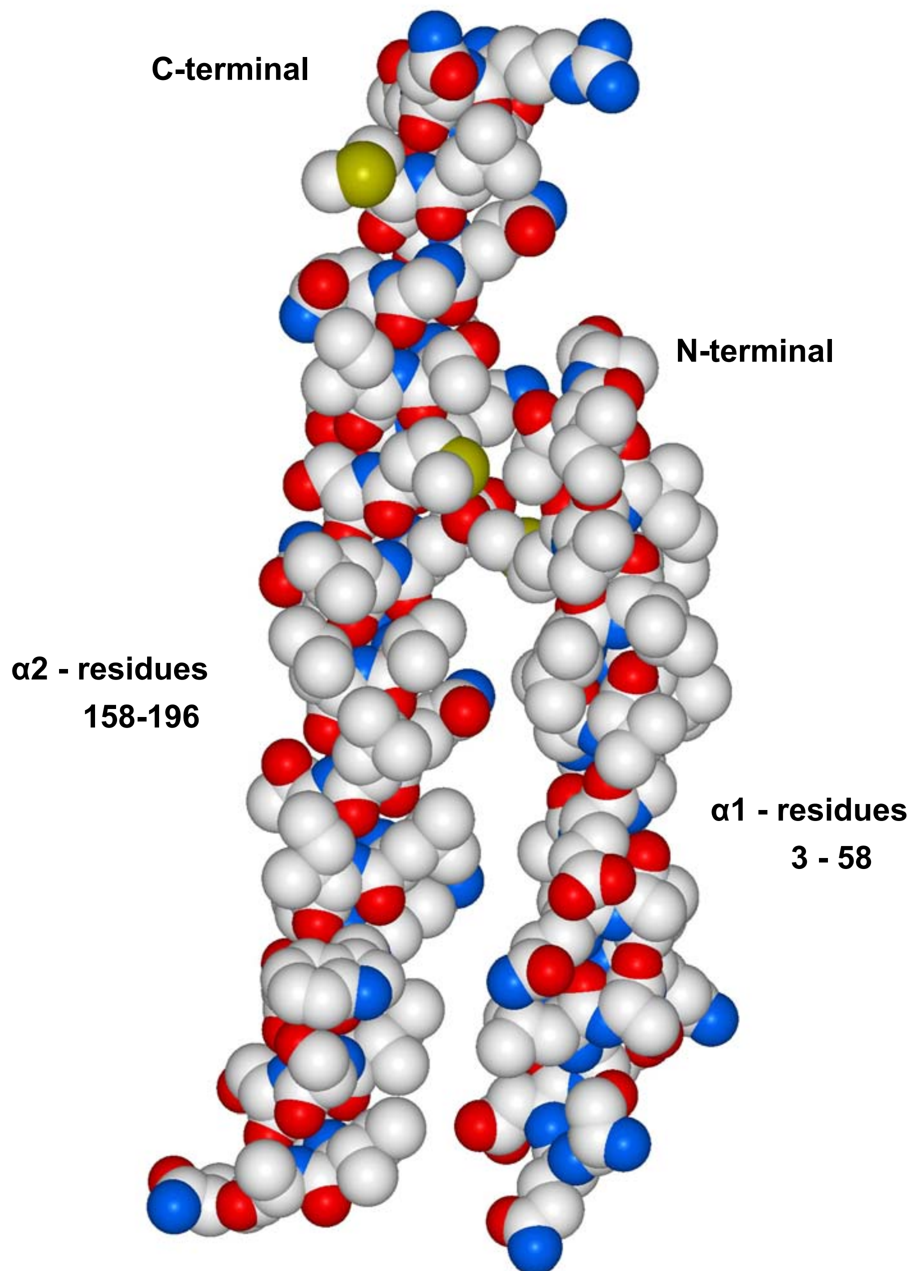

Figure 17: Space filling model of the SseB protein

All carbon atoms are colored light gray color, nitrogen -atoms are colored blue and oxygen atoms colored in red.

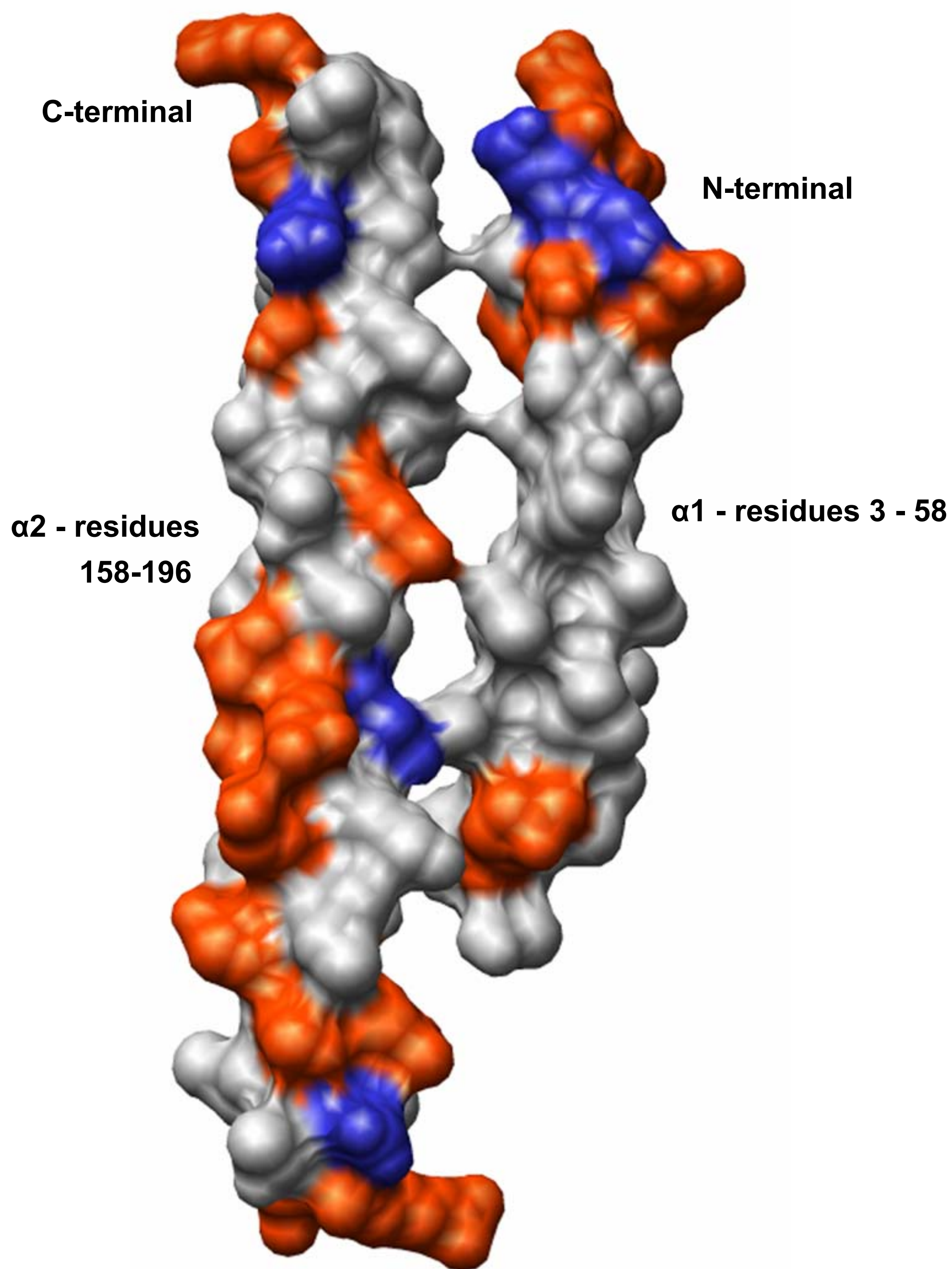

Figure 18: The surface area of SseB protein

The hydrophobic surface are colored light gray, basic residues are colored by blue and acidic residues are colored red.

The surface area diagram clearly shows that in both helices most of the hydrophobic residues are point towards each other and stabilize through hydrophobic interaction. In contrast, most of the hydrophilic amino acids are exposed to the solvent surfaces. Recent studies show that translocon complex such as SseB, SseC and SseD forms as a donut like structure, which facilitate translocation of toxin proteins into the host cells (8). Based on the surface area it can be predicted that solvent exposed surface may interact with α-helix of other part of the molecule or another SseB molecule or other translocon protein molecule (SseC and SseD). However, to unravel this puzzle the complete structure of SseB or translocation complex is required.

The structure is composed of many alpha-helices that run vertically forming a donut shaped complex with SseC and SseD. This donut shaped complex is what forms a tunnel through which effector proteins can be translocated. Further experiments need to be carried out to determine a better structure of SseB as well as a complex of SseB, SseC, and SseD so that the structure of the entire translocon can be determined.

V. CONCLUSION

The aim of this project was to determine the crystal structure of SseB of *Salmonella typhimurium.* SseB protein crystallized in one condition and it diffracted at 3.8Å. We are further optimizing the crystal condition to get higher resolution data. In addition, Se-Met crystallization is also under progress. The other approaches include cleaving the His-tag from the SseB protein or crystallizing it without the tag. In addition, SseB can also be co-crystallized with other proteins such as SseA or SseC which may give better diffraction than SseB alone. Currently, the work is ongoing to obtain the SseB crystal without His-tag as well as to get a binary complex.

A partial model of SseB was built using the crystal structure of EspA of *E. coli.* The partial model has two α-helices and it is stabilized by hydrophobic interaction. The complete structure of SseB as well as a structure of a complex of SseA, SseB and SseC needs to be solved. This will provide a holistic understanding of the translocon complex and the role that it plays in the Type III Secretion System.

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2. Process of protein transport by the Type III secretion system. Ghosh, P. *Microbiology and Molecular Biology Reviews* (Dec. 2004), 771 – 795
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Grade Average: 3.99/4.0

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Graduated: May 2004
Grade Average: 4.0 (Salutatorian, Summa Cum Laude, Texas Scholar)

EXPERIENCE

Sacchettini Lab, Department of Biochemistry and Biophysics, Texas A&M University, College Station, Texas; Fall 2004, Spring 2005, Fall 2005, Spring 2006, Fall 2006. Spring 2007

Research Lab Assistant

- Make agar plates, culture media and solutions
- PCR, cloning, digestion, ligation, inoculation
- Extract DNA/plasmid
- Transformation, gel electrophoresis
- Grow cell cultures, express and purify protein
- Set up x-ray crystallization plates and screen for protein crystals

Wehrens Lab, Department of Molecular Physiology and Biophysics, Baylor College of Medicine, Houston, Texas; December 2006 – January 2007

Research Lab Assistant

- Digest mouse tails and extract DNA
- PCR
- Genotype mice
- Purify DNA and PCR products
- Observe experiments on mice

Medinet Family Care Clinic, Houston, Texas; June 2005 - August 2005

Medical Assistant

- Run an EKG, urinalysis, strep test, pulse oximetry, audiogram
- Triage and take vital signs
- Call in prescriptions
- Verify insurance and schedule appointments
- Shadow physicians and assist with minor procedures

Medical School Familiarization Program I & II, University of Texas Medical Branch, Galveston, Texas; June 2005, June 2006

Student

- Clinical rotation in the psychiatry
    - Review nurse's reports
    - Interview patients
    - Discuss patient progress with doctors and nurses
- Preparation for the MCAT
- Observe plastic surgery in Shriners Burns Hospital for Children
- Attend seminars by guest speakers
- Learn about the field of medicine and being a competitive applicant

VOLUNTEER EXPERIENCE

St. Joseph's Regional Health Center, College Station, Texas, Fall 2006

Emergency Room

- Triage and take vital signs, run an EKG, pulse oximetry
- Transport patients, specimens and assist nurses
- Stock all necessary supplies

HOSTS (Helping One Student To Succeed), College Station, Texas, Fall 2006

Mathematics Tutor

- Assist class with middle school math
- Provide one-on-one help with homework

West Houston Medical Center, Houston, Texas; Summer 2003

Volunteer Assistant to the Pharmacist and the Geriatric Psychiatrist

- Dispense and deliver medications
- Help prepare IVs
- Assist therapist, nurses, and patients

ACTIVITIES

Texas A&M Tuberculosis Awareness Group

Founder & President

- Wrote the constitution and established the organization
- Raise awareness about the disease
- Promote the need for funding for tuberculosis research
- Assist with the creation and the maintenance of a global TB website: www.webTB.org

Aggie Book Club

Co-founder & Vice President

- Wrote the constitution and established the organization
- Read and discuss novels, short stories, and poems
- Read books at elementary schools and hospitals
- Invite authors and guest speakers

Alpha Epsilon Delta (Pre-medical Honor Society)
Member

- Attend lectures and learn about medical school
- Community service

Phi Eta Sigma (National Honor Society)
Historian

- Assist with planning meetings and special events
- Take photographs at all events
- Make a scrapbook
- Community service

Hindu Students Council
Special Events Coordinator

- Organize events and festival celebrations
- Geeta study and prayers
- Plan meeting discussions and presentations
- Schedule guest speakers

Honors Students Council
Honors Scheduling Open House Committee

- Write invitations to professors to attend the Honors Scheduling Open House
- Interview professors
- Help organize the open house
- Help organize Honors Week and Parents Weekend

HONORS AND AWARDS

One of the four Texas A&M Students nominated for the Barry M. Goldwater Scholarship
Gathright Award for the most outstanding student in the College of Ag & Life Sciences
Gamma Sigma Delta most outstanding student award in the College of Ag & Life Sciences
Student Research Week – 2nd place in the Biological Sciences Category
Student Research Week – Interdisciplinary Research Recognition
Who's Who Among Students in American Universities and Colleges for 2006-2007
Honors Undergraduate Research Fellow

Biochemistry Research Fellow
Texas A&M Academic Excellence Award
Dean's Honor Roll
University Honors and Foundation Honors
Texas Aggie Scholar
Honors Incentive Award
President Clinton Academic Achievement Award
Governor Rick Perry Achievement Award

LANGUAGES

Fluently speak English, Gujarati and Hindi. Have conversational skills in Urdu with basic understanding of Spanish.