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Comparative Insilco structural characterization of anaerobic regulators involved in *Salmonella typhimurium* host interactions

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Abstract
Salmonella typhimurium is one important intracellular, gram negative, non-spore forming pathogen causing food poisoning in human worldwide. During its pathogenesis, it encounters various anaerobic conditions, which is mediated by different regulators. NarL, narG, ssrB and FNR are important anaerobic regulators; affect the pathogenesis in the host cell. So there is a need of physiochemical and structural characterization of these proteins. So in this study, these anaerobic regulators proteins were characterized physiochemically by EXPASY Protoparam bioinformatics tool. Further the secondary and three dimensional structures were predicted through GORIV and SWISS Homology Modeling software tool respectively. All these proteins were found as stable, soluble, hydrophilic in nature. Further, NarL, ssrB and FNR showed a greater number of alpha helixes followed by random coils where as NarG contains large number of random coils. The three-dimensional structure of all NarL, NarG, ssrB and FNR showed that all proteins exist in monomer with RMSD value in least difference. So this study will provide a better platform for researcher to develop suitable therapeutics against these anaerobic regulators in *Salmonella typhimurium* infection.

Keywords: *Salmonella typhimurium*, anaerobic regulators, SWISS homology modeling

Introduction
Now a day's *Salmonella* infection is considered a major zoonotic disease as more than 300,000 people are thought to die from it every year, largely in developing nations [1]. It is one important cause of food poisoning characterized by gastroenteritis, bacteraemia, and enteric fever contributing major economic burden worldwide [2]. *Salmonella typhimurium* is a nontyphoidal, gram negative, rod shaped, facultative, Enterobacteriaceae serovar with broad host range including human most often causes self-limiting gastroenteritis [3]. During pathogenesis, *Salmonella typhimurium* encounter intestine and switch over from aerobic to anaerobic metabolism which is mediated by various anaerobic regulators and their operon [4]. Nitrate anaerobic regulator L (NarL), Fumarate nitrate reductase regulator (Fnr), nitrate reductase (NarG), secretion system regulator (ssrB) are important regulators controls the pathogenesis of salmonella in anaerobic condition [5]. NarL is one important component of NarL/NarX dual regulatory complexes regulates the nitrate/nitrite redox reaction, modulates the anaerobic respiration [6]. FNR is a heterodimeric DNA binding iron sulfur metalloprotein that senses the fluctuations in oxygen tension in the surrounding and gets activated in anaerobic environment [7]. Further, narG is one important component of narGHI operon, control the expression of nitrate reductase which mediates the conversion of nitrate to nitrite [8]. SsrB is involved in a molecular regulatory switch that aids in Salmonella transition to an intracellular lifestyle. It induces the expression of the SPI-2 genes and other genes located outside SPI-2, which are required for survival and replication, while simultaneously repressing the expression of the hilD and hilA SPI-1 regulatory genes, and the flagella-based motility genes [9]. By viewing important of these regulators in pathogenesis of *salmonella*, these regulators need to be structurally and physiochemical characterized at molecular level for discovery of natural inhibitors and drugs against salmonellosis. Further, advancement of computational biology and bioinformatics tools helps in thorough characterization of protein at Insilco level [10]. Further the structural and physiochemical characterization of these anaerobic regulators are still unclear, due to lack of experimental 3 D structure. In this study we have explored the model structure of all the above proteins through Insilco platform and that could

be targeted for discovery of new therapeutic regimen for treatment of salmonellosis in nearest future.

Materials and Methods

Retrieval of sequences

The amino acid sequence of aforesaid proteins of *Salmonella typhimurium* such as narL protein (Accession Number: NP_460723.1), narG protein (Accession Number: NP_460720.1), ssrB protein (Accession Number: NP_460356.1) and fnr (Accession Number: NP_460619.3) were retrieved in FASTA format from National Center for Biotechnology Information (NCBI) database.

Physiochemical characteristics

The physicochemical characteristics of the above proteins, including their amino acid composition, molecular weight, theoretical pI, extinction coefficient, absorbance, half-life, stability index, aliphatic index, and grand average of hydropathicity (GRAVY) were calculated using ProtParam characterization tools under the Expert Protein Analysis System (Expasy) server [11]. The solubility and sub cellular localization were also predicted by SOSUI (http://harrier.nagahama-i-bio.ac.jp/sosui/) and WoLFPSORT https://www.genscript.com/ wolf-psort.html) software respectively.

Protein disorder prediction

The disordeness of all the four proteins were predicted by IUPred2A [12] and PONDR [13] server.

Prediction of secondary structure and analysis

The secondary structure of all the above proteins (Alpha helix, Beta Bridge, Beta turn, extended strand and Random coil) were estimated by using GORIV secondary structure prediction method [14].

Tertiary structural characterization

SWISS Modeling was performed via SWISS-MODEL server (ProMod3 3.0.0) that aligns the input NarL, NarG, ssrB and FNR protein with preexisting templates (as shown in Table 1) to generate 3 D structure of our target proteins [15]. Lastly, structural analysis was performed and model figures were generated by Biovia Discovery Studio Visualizer, 2021 [16]. DALI server was used for alignment of generated models with their templates to determine the Z value and the Root Mean Square Deviation (RMSD) value [17].

Result and Discussions

As animal husbandry contributes 4% to India's GDP, it is considered as the principal alternative income generating avenue for the farming community but the emergence of various bacterial diseases hampers the economic return to the farmer [18]. More over *Salmonella typhimurium*, a zoonotic organism, is solely responsible for majority of foodborne nontyphoidal salmonellosis (NTS). NTS is asymptomatic in majority of population, while it can be associated with fever, colitis, diarrhea and sometimes splenomegaly. The cases of NTS gastroenteritis in 2010 was 93 million worldwide with an estimated death of 155,000 Salmonella [19]. Further, anaerobic regulators such as narL, narG, ssrB, fnr mediate its pathogenesis in anaerobic condition.

In this study, the results of physiochemical characteristics of all four proteins were shown in Table No 1. It was found that the amino acid compositions of NarL, ssrB and FNR did not varied significantly, but the amino acid composition of narG showed highest number of difference as narG is a subunit of narGHI operon which encodes nitrate reductase enzyme [20]. The value theoretical pI revealed that all the anaerobic regulator protein of *Salmonella typhimurium* are acidic in nature except FNR protein which is basic in nature due to presence large amount of argininine, histidine and lysine aminoacids. The extinction coefficient was found higher in narG and ssrB protein than other proteins, inferred that these two proteins have higher lambda max than other. Further the instability index, which is an indicator of stability of protein, showed that all four proteins are stable in nature as the value is less than 40 [21]. The aliphatic index which indicates relative volume of aliphatic side chain, were found higher in all four proteins inferred that all these proteins are thermostable in nature. However, the GRAVY value was found less than zero interpreted that, all four proteins are hydrophilic in nature due to presence of more polar amino acids in their composition [22].

Table 1: Showing the physiochemical parameters of anaerobic regulator proteins of *Salmonella typhimurium*

	NarL protein	NarG protein	ssrB protein	FNR protein
Accession Number	NP_460723.1	NP_460720.1	NP_460356.1	NP_460619.3
No. of Amino acids	216	1247	212	264
Molecular Weight	23.86 kDa	140.68 kDa	24.35 kDa	29.48 kDa
pI	5.60	6.24	7.12	8.77
Extinction coefficient	6990	293190	26025	9190
Instability Index	32.20	31.77	31.15	39.30
Aliphatic Index	109.26	71.76	116.84	91.70
GRAVY	-0.144	-0.535	-0.061	-0.239

The SOSUI results of this study showed that all the proteins are soluble and cytoplasmic in nature (shown in Table 2) which can be interpreted that all the aforesaid proteins regulate the pathogenesis of *Salmonella typhimurium* by binding with the DNA during transcription [23].

Table 2: Showing the SOSUI results anaerobic regulator proteins

	Type of protein	Location of Protein
NarL	Soluble	Cytoplasmic
NarG	Soluble	Cytoplasmic
ssrB	Soluble	Cytoplasmic
FNR	Soluble	Cytoplasmic

The secondary structural characterization of all these four proteins (Showed in Table 3) revealed that, all four proteins contain more abundance of random coils than the alfa helix whereas ssrB contains more percentage of alpha helix than random coils. It might be due to presence of more specific and stabilizing interactions in ssrB protein that makes it secretory and more compact protein [24].

Table 3: Showing the composition of different secondary structure of anaerobic regulator proteins in *Salmonella typhimurium*

	NarL	NarG	ssrB	FNR
Alpha helix (%)	53.24	29.43	52.36	42.80
Extended strands (%)	8.80	20.05	13.21	15.91
Random coil (%)	37.96	50.52	34.43	41.29

The model structures of all four proteins derived from Swiss Homology Modeling were shown in Figure 1. All the models were generated by alignment with various templates considering various parameters such percentage of identity and resolution (shown in Table 4).

Fig 1: Model structure of NarL protein

Fig 2: Model structure of NarG protein

Fig 3: Model structure of ssrB protein

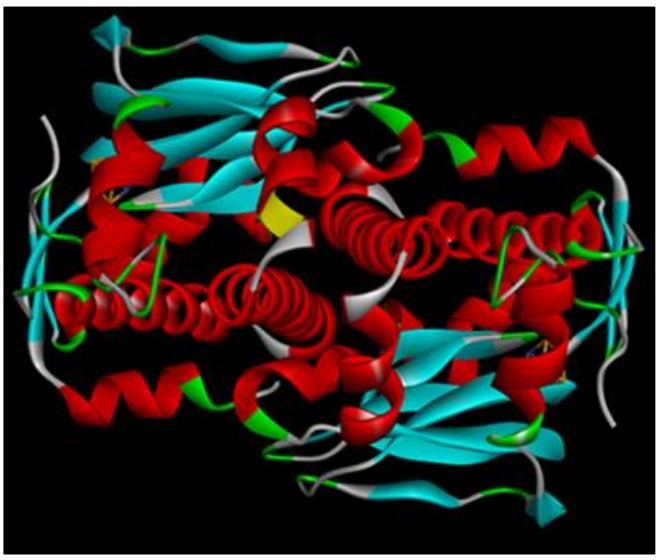

Fig 4: Model structure of FRB protein

Table 4: Showing the various parameters of protein models

	GMQE	Identity (%)	Resolution	Template	Z score	RMSD Value
NarL Model	0.92	96.73	2.40 °A	1rnl.1.A	38.9	0.1 Å
SsrB Model	0.62	94.21	2.20 °A	3c3w.1.A	35.6	0.2 Å
FNR Model	0.87	87.08	2.6°A	5e44.1.A	37.7	0.6 Å
NarG Model	0.90	95.27	2.8°A	3ir6.1.A	36.7	0.5

The best model of all proteins with lowest Z score was considered and it was seen that all the proteins exist in monomer. The Z value and the root mean square deviation (RMSD) between all models with their template showed minute difference i. e for NarL (0.1Å, 38.9), ssrB (0.2 Å, 35.6), FNR (0.6 Å. 37.7), NarG (0.5 Å. 36.7) were found. So it was interpreted that the models generated by Swiss modeling could be reliable for downstream structure analysis. This study also resulted that all proteins have various type of disorder region in their structure shown in Fig 5 to 8 which might be responsible for different types of protein protein and protein nucleic acid interactions. The disordered region also consists of various type domain regions shown in Table 5 that might be responsible for their biological activities.

Table 5: Conserved Domain of anaerobic regulatory proteins of *Salmonella typhimurium*

Type of proteins	Position	Type of Domains
NarL	8-121	Response regulatory Domain
	154-210	GerE domain
NarG	3-40	Nitarate reductase alfa unit N
	108-833	Molybdopterin
	1087-1206	Molybdopterin binding domain
SsrB	6-118	Response regulatory Domain
	148-204	GerE domain
FNR	63-149	cNMP binding domain
	182-256	HTH_crp_2

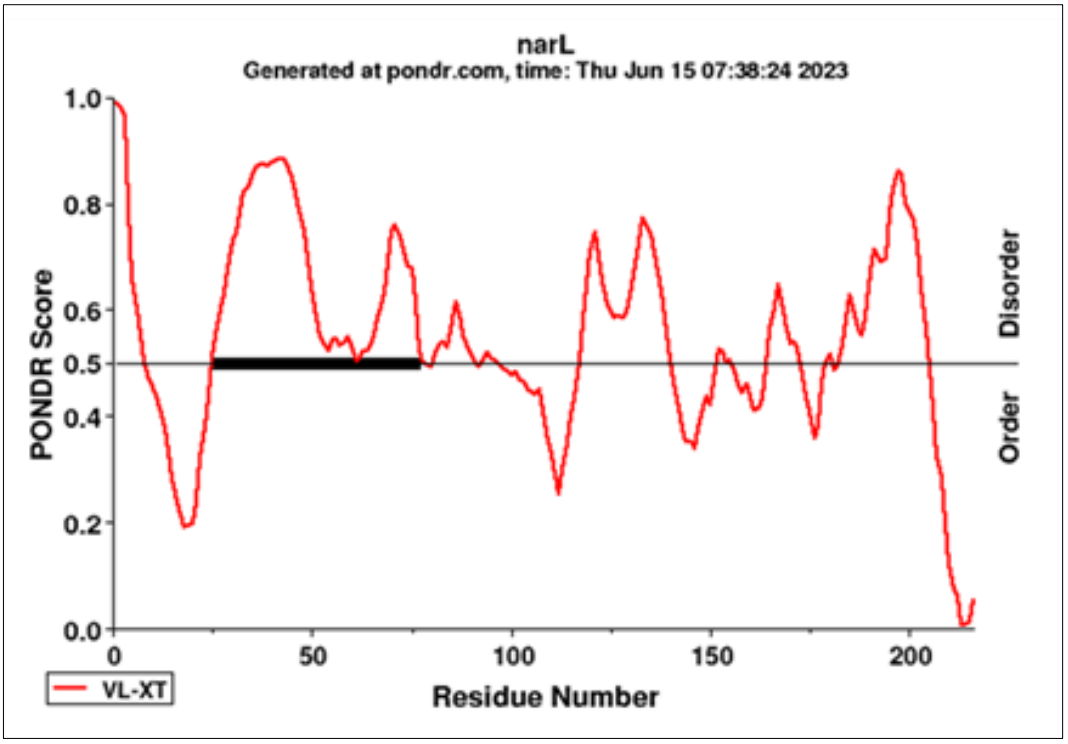

Fig 5A: NarL Protein Disorderness by PONDR Server

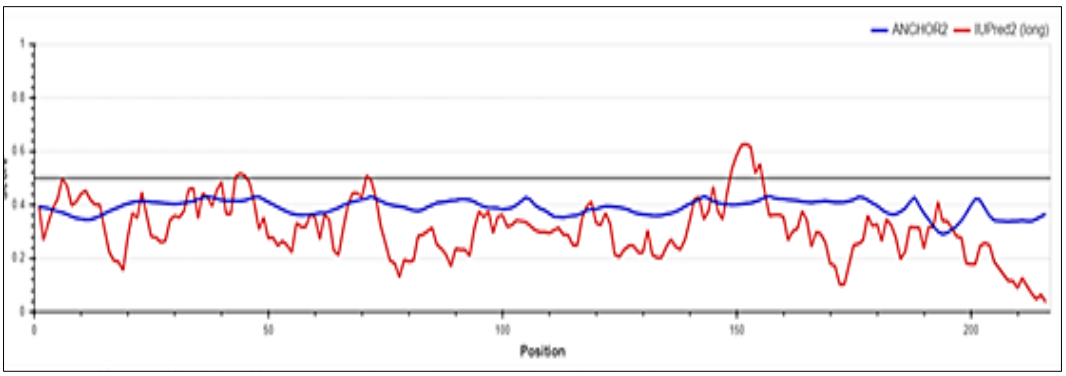

Fig 5B: NarL Protein Disorderness by IUPred2A Server

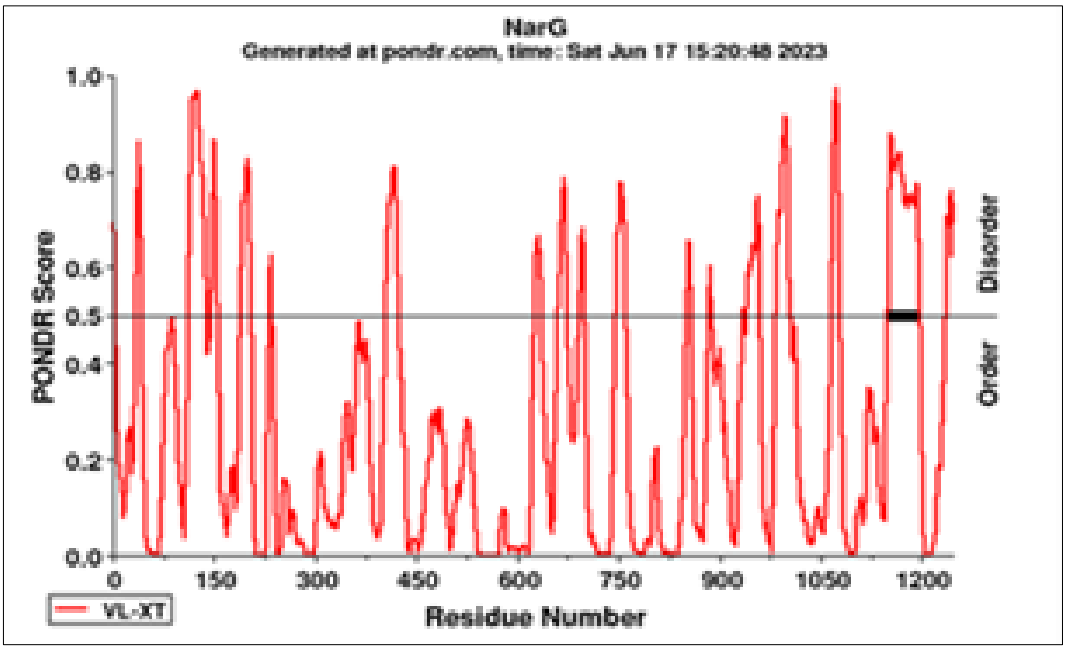

Fig 6A: NarG Protein Disorderness by PONDR Server

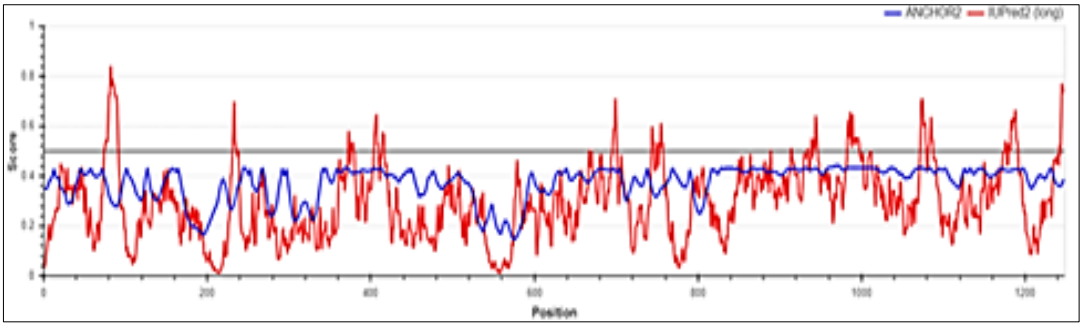

Fig 6b: NarG Protein Disorderness by IUPred2A Server

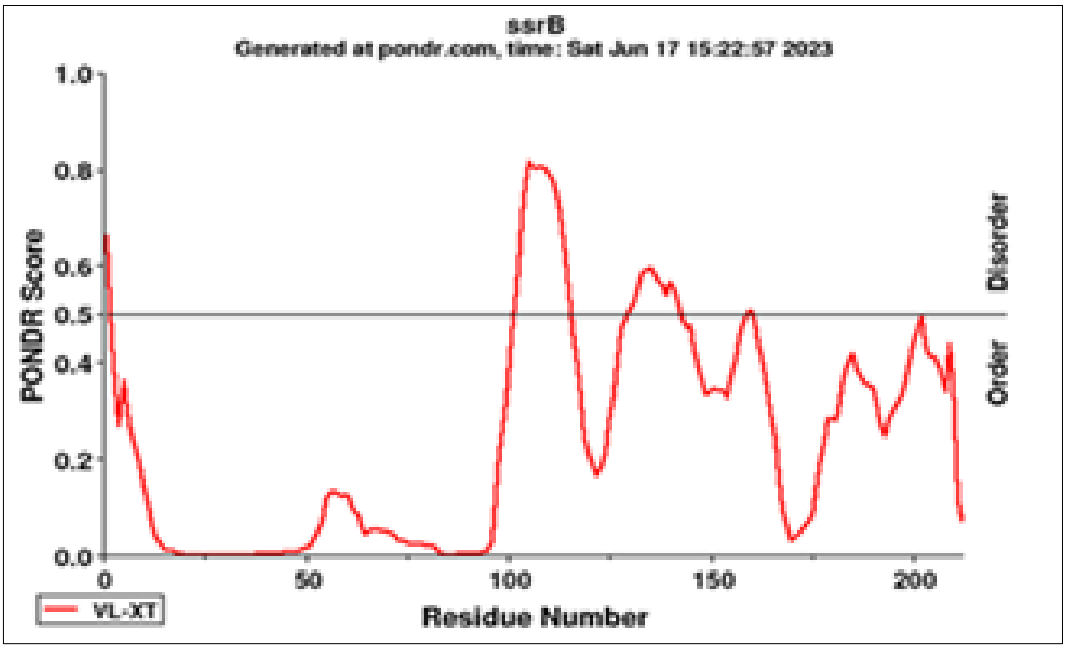

Fig 7A: ssrB Protein Disorderness by PONDR Server

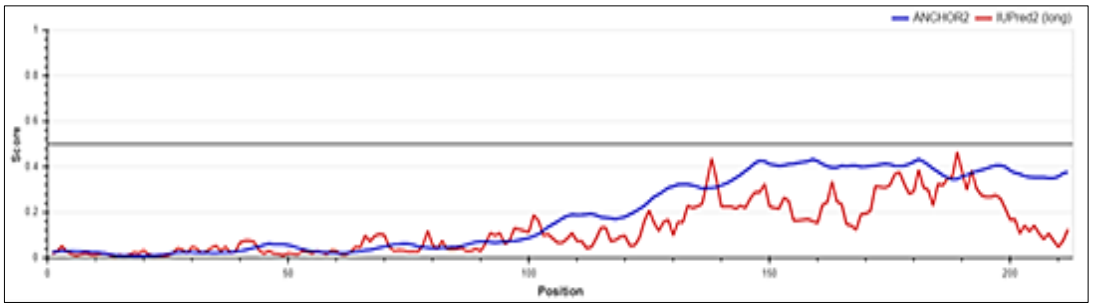

Fig 7b: SSRB Protein Disorderness by IUPred2A Server

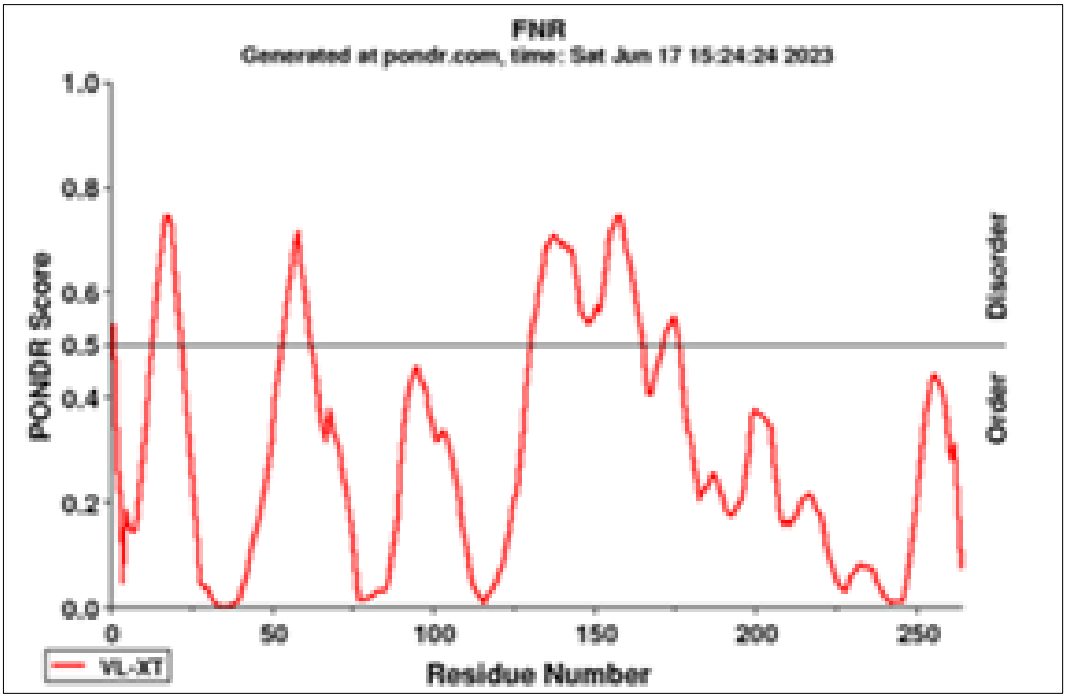

Fig 8A: FNR Protein Disorderness by PONDR Server

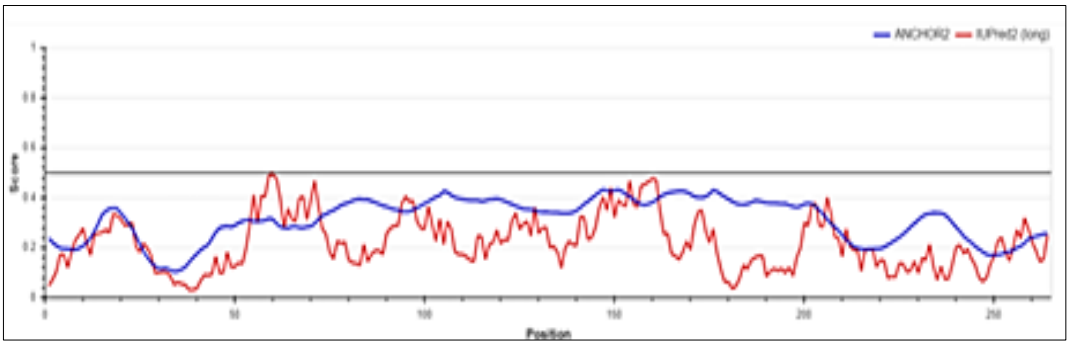

Fig 8b: FNR Protein Disorderness by IUPred2A Server

Conclusion

This work concludes that these anaerobic regulator proteins are crucial for *Salmonella typhimurium* pathogenesis in anaerobic condition. These proteins might be good therapeutic target against this bacterial infection. The development of possible bioinformatics tools and databases would provide a better platform for characterizing these proteins in terms of physiochemical and structural parameters. Therefore, this research will aid in the creation of potentially successful and efficient targets for the *Salmonella typhimurium* through molecular docking in nearest future.

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