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Some Transmembrane Proteins of Salmonella Typhimurium Are Potential Drug Targets: An In Silico Analysis

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Abstract: Salmonella sp. is a globally prevalent organism responsible for causing salmonellosis, a foodborne illness. Salmonella Typhimurium represents a type of Salmonella sp. not associated with typhoid fever but capable of causing stomach and intestinal inflammation or severe infection. In the present study, 150 uncharacterized proteins of S. Typhimurium were randomly selected from UniProtKB and analyzed using TMHMM, PROSITE, STRING, DEG, and BLASTp. Results indicated that 32 uncharacterized proteins (21%) were predicted to be transmembrane proteins involved in various biological pathways. Among them, 30 transmembrane proteins were predicted to be essential and non-host homologous. This study's findings suggest their potential as drug targets against salmonellosis.

Keywords: Salmonella; transmembrane protein; drug targets; in silico

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1. Introduction

Salmonella sp. is the worldwide pathogen that causes salmonellosis, a foodborne disease. Salmonella typhimurium is a nontyphoidal salmonella that can cause gastroenteritis or invasive disease. Invasive nontyphoidal salmonellosis often involves immunocompromised individuals, especially those who suffered with HIV infection. Salmonella sp. infection can be transmitted either by contact or by ingestion of contaminated food. The presence of multidrug resistance Salmonella sp. is a serious issue for disease spread. Salmonella enterica serovar has been divided into two groups which is typhoidal and non-typhoidal (NTS) (Okoro et al., 2015). In general, Salmonella sp. is a Gram-negative, facultative anaerobic bacterium under Phylum Proteobacteria with peritrichous flagella for locomotion. It also has fimbriae that helps S. typhimurium adhere to cell surfaces, increasing the probability of disease production. Additionally, S. typhimurium has specialized sex pili for genetic information exchange between cells. It inhabits the intestinal tracts of humans and animals, especially poultry and cattle. The infection can be transmitted through person-to-person contact via saliva or mouth-to-mouth contact with an infected person (Fernandes et al., 2016). S. Typhimurium invades the intestinal mucosa, multiplies within vesicles inside cells, and crosses epithelial cell membranes to enter the lymphatic system and bloodstream, causing acute intestinal inflammation in humans (Hapfelmeier & Hardt, 2005). Biofilm formation by S. Typhimurium has been reported over many decades (Armon et al. 1997; Lapidot et al. 2006; Yahya et al. 2017; Johari et al. 2023).

Transmembrane proteins confer particular properties to the membrane, including signal transduction and the transport of ions or small molecules across it. Some transmembrane proteins bind to hormone or neurotransmitter receptors, altering their structure and triggering specific reactions. Additionally, they selectively transport substances such as ions or molecules across the membrane, establishing concentration gradients or energy potentials between intracellular and extracellular environments via active or passive transport. Due to their unique features,

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transmembrane proteins are under extensive research for various applications in sensors, screening, water purification, and energy harvesting (Ryu et al., 2019). Identifying transmembrane proteins in pathogenic microorganisms involves employing bioinformatic tools and computational approaches to predict and analyze protein structures and functions. Sequence-based bioinformatics tools are utilized to detect potential transmembrane domains within protein sequences, employing algorithms like Hidden Markov Models (HMMs) and Position-Specific Scoring Matrices (PSSMs) to recognize characteristic patterns indicative of transmembrane regions (Khan & Uddin 2022). Subsequently, structural bioinformatics tools are employed to model the three-dimensional structure of identified proteins, enabling visualization of transmembrane domains and their orientation within the lipid bilayer.

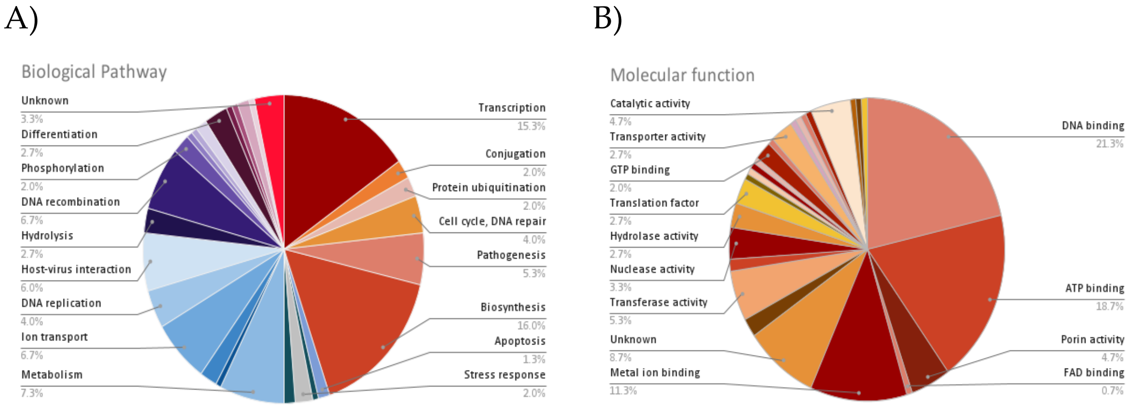
Bioinformatics plays a crucial role in identifying drug targets in Salmonella through diverse computational methods and data analysis techniques. It facilitates the analysis of genomic and proteomic data from Salmonella strains, aiding in pinpointing potential drug targets (Khan & Uddin 2022). By comparing genomes of drug-resistant strains with susceptible ones, bioinformatics detects genetic variations linked to resistance, thus highlighting potential intervention targets (Jalal et al. 2021). Additionally, bioinformatic tools predict the function and structure of proteins encoded by Salmonella genes, assisting in selecting targets with druggable properties. This study aims to analyze uncharacterized proteins of S. typhimurium for potential drug targets.

2. Materials and Methods

A total of 150 uncharacterized proteins of S. Typhimurium were randomly selected and retrieved from UniprotKB database. They were analyzed using TMHMM, PROSITE, STRING, DEG and BLASTp.

3. Results

Figure 1 shows the classification of uncharacterized proteins from Salmonella Typhimurium based on their biological pathways, molecular functions, subcellular localization, and identification as transmembrane proteins. The majority of the proteins are involved in biosynthesis (16%) and DNA binding (21.3%) and located in the cytoplasm (64.7%) for their biological pathway and subcellular localization, respectively. Only 21.3% of the uncharacterized proteins were predicted to be transmembrane proteins.



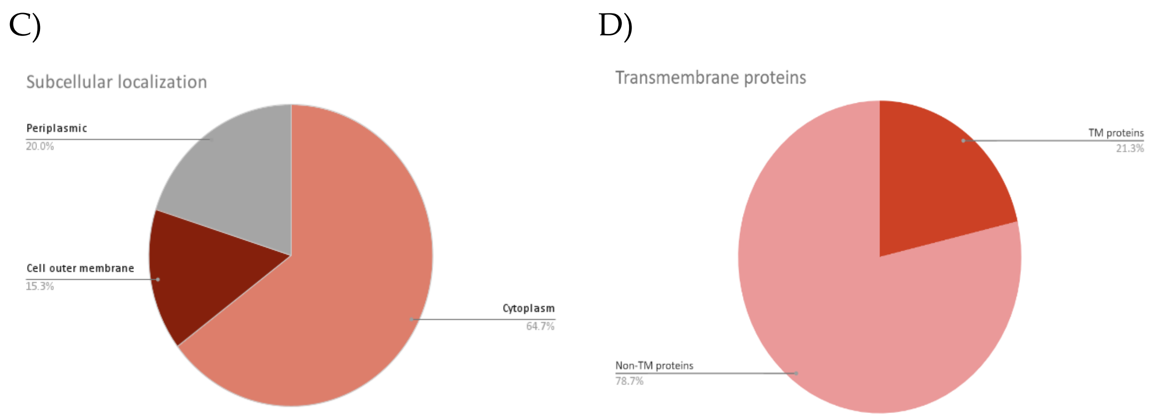


Figure 1. Classification of uncharacterized proteins of S. Typhimurium. A) Biological pathway; B) Molecular function; C) Subcellular localization; D) Transmembrane proteins.

Table 1 lists the identified transmembrane proteins from S. Typhimurium along with details about the number of transmembrane domains, their predicted biological pathways, molecular functions, and subcellular localization. Protein A0A2J0RKS1 showed the highest number of predicted transmembrane domains.

Table 1. Identified transmembrane proteins of S. Typhimurium.

Accession	No. of TM domain	Biological pathway	Molecular function	Subcellular localization
A0A2J0RKS1	9	Conjugation	Porin activity	Cell outer membrane
A0A1P8DMP4	1	Unknown	ATP binding	Plasma membrane
A0A3Z7JEV9	1	DNA repair	DNA ligase activity	Cytosol
A0A2J0RFX8	1	Catabolic process	D-aminoacyl-tRNA deacylase activity	Cytoplasm
A0A2J0RDC1	1	Translocation	Unknown	Nucleus
A0A3T3ZZV4	1	tRNA processing	ATP binding	Cytoplasm
A0A3V6H1D5	1	Pathogenesis	Unknown	Plasma membrane
A0A0D6HCE5	2	Lipoprotein biosynthesis	phosphatidylglycerol-prolipoprotein diacylglyceryl transferase activity	Plasma membrane
A0A2J0RDS6	1	Host-virus interaction	Unknown	Host membrane
A0A3V7XF93	1	Carbohydrate metabolic process	Carbohydrate binding	Extracellular region or secreted
A0A3V7X7Z5	2	Host-virus interaction	ATP binding	Cell membrane
A0A5Z7LRR5	4	Proteolysis	Metal ion binding	Cell membrane
A0A5Z7LRA6	1	Cell cycle	ATP binding	Nucleus
A0A0F7J9G5	1	DNA packaging	Metal ion binding	viral terminase complex

A0A0F7JGQ1	1	Cell adhesion	Protein-containing complex binding	Extracellular region or secreted
A0A0F7JFI2	1	Differentiation	Zinc ion binding	Extracellular region or secreted
A0A0F7JDX1	2	Establishment of competence for transformation	Unknown	Plasma membrane
A0A5Y2HQY6	1	Amino acid biosynthesis	ATP binding	Cytoplasm
A0A0F7J982	1	Protein biosynthesis	GTP binding	Cytoplasm
A0A6C8WQJ4	3	inorganic anion transport	Chloride channel activity	Plasma membrane
A0A735ZTN8	7	Transmembrane transport	Porin activity	Plasma membrane
A0A731QU75	2	Chemical synaptic transmission	G-protein coupled receptor activity [serotonin]	Plasma membrane
A0A607WTJ3	6	Phospholipid biosynthetic process	Transferase activity	Plasma membrane
A0A705WX69	1	Oxidative phosphorylation	Translocase	Plasma membrane
A0A707YZC5	1	Cytochrome complex assembly	heme transmembrane transporter activity	Plasma membrane
A0A706T8K4	2	Transmembrane transport	transmembrane transporter activity	Vacuole
A0A717VZE3	1	DNA replication	DNA binding	Plasma membrane
A0A736JL85	2	Folate biosynthesis	metal ion binding	Inner membrane
A0A610AT56	3	Phospholipid biosynthetic process	Transferase activity	Plasma membrane
A0A705WZ57	1	Transmembrane transport	Transmembrane transporter activity	Plasma membrane
A0A701H8E2	4	Cytochrome c-type biogenesis	Heme binding	Plasma membrane
A0A7G2DIQ3	2	Viral tail assembly	Unknown	Host cell cytoplasm

Table 2 summarizes the BLASTp analysis results, showing the presence or absence of homologous proteins in various hosts (human, cattle, sheep, goat, and horses) for the identified transmembrane proteins from S. Typhimurium. Most of the proteins showed no homolog in the tested hosts, except for A0A0F7JDX1, which had a homolog in sheep, and A0A717VZE3, which had a homolog in humans. The protein A0A610AT56 is marked as a non-essential protein. A total of 29 transmembrane proteins were predicted to be essential and non-host homologous.

Table 2. List of essential and non-host homologous proteins of Salmonella Typhimurium.

Protein	**Human**	**Cattle**	**Sheep**	**Goat**	**Horses**
A0A2J0RKS1	0	0	0	0	0
A0A1P8DMP4	0	0	0	0	0
A0A3Z7JEV9	0	0	0	0	0
A0A2J0RFX8	0	0	0	0	0

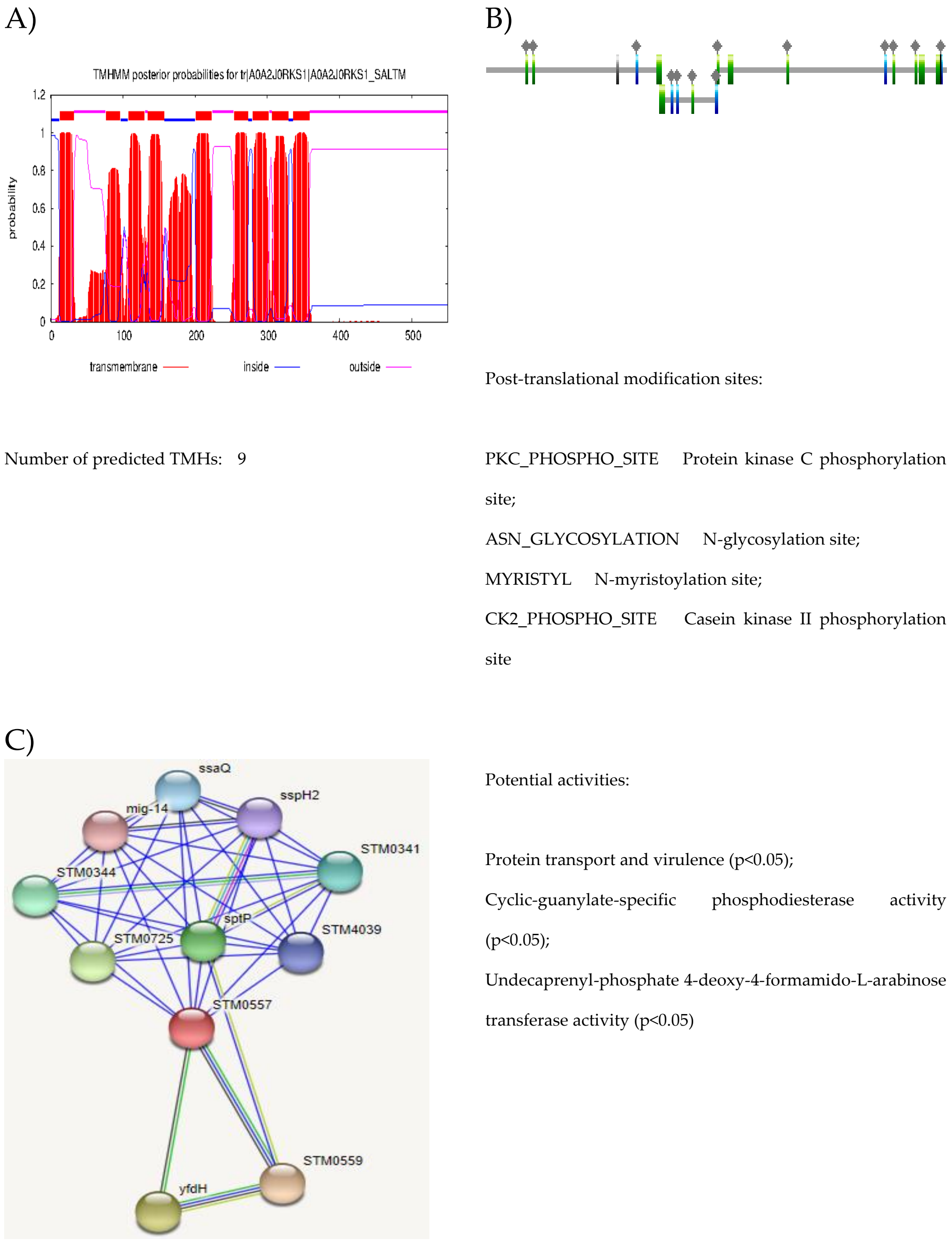
A0A2J0RDC1	0	0	0	0	0
A0A3T3ZZV4	0	0	0	0	0
A0A3V6H1D5	0	0	0	0	0
A0A0D6HCE5	0	0	0	0	0
A0A2J0RDS6	0	0	0	0	0
A0A3V7XF93	0	0	0	0	0
A0A3V7X7Z5	0	0	0	0	0
A0A5Z7LRR5	0	0	0	0	0
A0A5Z7LRA6	0	0	0	0	0
A0A0F7J9G5	0	0	0	0	0
A0A0F7JGQ1	0	0	0	0	0
A0A0F7JFI2	0	0	0	0	0
A0A0F7JDX1	0	0	1	0	0
A0A5Y2HQY6	0	0	0	0	0
A0A0F7J982	0	0	0	0	0
A0A6C8WQJ4	0	0	0	0	0
A0A735ZTN8	0	0	0	0	0
A0A731QU75	0	0	0	0	0
A0A607WTJ3	0	0	0	0	0
A0A705WX69	0	0	0	0	0
A0A707YZC5	0	0	0	0	0
A0A706T8K4	0	0	0	0	0
A0A717VZE3	1	0	0	0	0
A0A736JL85	0	0	0	0	0
A0A610AT56 *	0	0	0	0	0
A0A705WZ57	0	0	0	0	0
A0A701H8E2	0	0	0	0	0
A0A7G2DIQ3	0	0	0	0	0

(0) = absence homologues

(1) = presence homologue

(*) refer to non-essential protein.

Figure 2A shows the number of predicted transmembrane helices (TMHs) for the protein A0A2J0RKS1, which is 9. Figure 2B displays the potential post-translational modification sites identified in the protein A0A2J0RKS1, including protein kinase C phosphorylation sites, N-glycosylation sites, and N-myristoylation sites. This network visualization represents the potential activities and functional linkages of the protein A0A2J0RKS1 based on protein-protein interactions (Figure 2C). The predicted activities include protein transport, virulence, cyclic-guanylate-specific phosphodiesterase activity, and undecaprenyl-phosphate 4-deoxy-4-formamido-L-arabinose transferase activity.



Number of predicted TMHs: 9

Post-translational modification sites:

PKC_PHOSPHO_SITE	Protein kinase C phosphorylation site;
ASN_GLYCOSYLATION	N-glycosylation site;
MYRISTYL	N-myristoylation site;
CK2_PHOSPHO_SITE	Casein kinase II phosphorylation site

Potential activities:

Protein transport and virulence ($p<0.05$);
Cyclic-guanylate-specific phosphodiesterase activity ($p<0.05$);
Undecaprenyl-phosphate 4-deoxy-4-formamido-L-arabinose transferase activity ($p<0.05$)

Figure 2. A) Transmembrane domains of A0A2J0RKS1; B) Functional motifs of A0A2J0RKS1; C) Protein-protein interaction network of A0A2J0RKS1 (STM0557).

4. Discussion

Studying proteins in pathogens, particularly those involved in biofilm formation, is crucial as biofilms are complex communities of microorganisms encased in a self-produced extracellular matrix, enabling bacteria to adhere to surfaces and form resilient structures resistant to antimicrobials and host immune responses (Yaacob et al. 2021; Kamaruzzaman et al. 2022; Johari et al. 2023). Understanding transmembrane proteins may provide insight into their functions and therapeutic potential (Attwood & Schiöth 2021; Saches et al. 2021). Most identified transmembrane proteins of S. Typhimurium were predicted to be essential and non-host homologous to humans, cattle, sheep, goats, and horses. These proteins, crucial for bacterial survival and virulence but absent in host organisms, are potential therapeutic targets (Yahya et al. 2014, Othman and Yahya 2019; Nogueira et al. 2021). Protein A0A2J0RKS1, with the highest number of transmembrane domains, was predicted to have numerous functional linkages, establishing it as a significant hub protein in S. Typhimurium. Identifying essential hub proteins in pathogenic microorganisms via protein-protein interaction networks has been reported in several studies (Abd Rashid et al. 2022; Isa et al. 2022; Zulkiply et al. 2022; Bajire et al. 2023; Nithya et al. 2023).

5. Conclusions

We have shown that numerous uncharacterized proteins of S. Typhimurium hold promise as drug targets. Thirty transmembrane proteins identified in this study are essential and non-host homologous. Ongoing analysis of these transmembrane proteins is crucial to aid in the creation of effective drugs targeting Salmonella infection.

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