SCIENCE LETTERS

ISSN: 1675-7785
eISSN: 2682-8626

Available online at
https://scilett-fsg.uitm.edu.my/

Science Letters 19(1) 2025, 104 – 116.

Science Letters

In Silico Identification of Essential Transmembrane Proteins in *Salmonella typhimurium* as Potential Drug and Vaccine Targets

Izzati Nasuha Mohd Samsidi[1], Nawal Zulkiply[1], Anati Abd Rashid Syaida[1], Mohd Fakharul Zaman Raja Yahya[1,2]*

[1]*Faculty of Applied Sciences, Universiti Teknologi MARA (UiTM), 40450 Shah Alam, Selangor, Malaysia*
[2]*Integrative Pharmacogenomics Institute (iPROMISE), 42300 Bandar Puncak Alam, Selangor, Malaysia*

ARTICLE INFO

Article history:
Received 14 October 2024
Revised 15 December 2024
Accepted 18 December 2024
Published 30 January 2025

Keywords:
Drug targets
In silico
Salmonella
Transmembrane protein

DOI: 10.24191/scl.v19i1.6355

ABSTRACT

Salmonella sp. is a globally prevalent organism responsible for causing salmonellosis, a foodborne illness. *Salmonella typhimurium* is a strain of *Salmonella* sp. that is not associated with typhoid fever but can cause gastrointestinal inflammation and severe infection. Despite advancements in genomic and proteomic technologies, several proteins identified in *S. typhimurium* remain uncharacterized. Thus, the objective of the present study was to characterize the transmembrane proteins of *S. typhimurium* that could serve as potential drug and vaccine targets. In this study, 150 uncharacterized proteins from *S. typhimurium* were randomly selected from UniProtKB and analyzed using PSORTb V3.0.3 and TMHMM. Identified transmembrane proteins were further analyzed using DEG, BLASTp, ProtParam, ScanProsite, STRING 12.0, and VaxiJen 2.0. The results indicated that 32 uncharacterized proteins (21%) were predicted to be transmembrane proteins involved in various biological pathways. Among these transmembrane proteins, protein A0A2J0RKS1 was predicted to be essential, antigenic, and non-host homologous, suggesting its potential as a drug and vaccine target for combating salmonellosis. This study underscores the potential of computational biology in drug target discovery, particularly against pathogens like *S. typhimurium*.

* Corresponding author. *E-mail address*: fakharulzaman@uitm.edu.my

INTRODUCTION

Salmonella sp. is a globally prevalent pathogen that causes salmonellosis, a foodborne disease. *Salmonella typhimurium* is a non-typhoidal strain of Salmonella that can cause gastroenteritis or invasive disease. Invasive non-typhoidal salmonellosis often affects immunocompromised individuals, particularly

those with HIV infection. Salmonella infection can be transmitted through direct contact or ingestion of contaminated food. The emergence of multidrug-resistant Salmonella sp. is a significant concern for disease spread. *Salmonella enterica* serovars are classified into typhoidal and non-typhoidal (NTS) (Okoro et al., 2015). *Salmonella sp.* is a Gram-negative, facultative anaerobic bacterium in the phylum Proteobacteria, possessing peritrichous flagella for motility. It also has fimbriae that allow *S. typhimurium* to adhere to cell surfaces, increasing the likelihood of infection. Additionally, *S. typhimurium* possesses specialized sex pili for exchanging genetic material between cells. *Salmonella* inhabits the intestinal tracts of humans and animals, particularly poultry and cattle. Transmission can occur through person-to-person contact via saliva or mouth-to-mouth contact with an infected individual (Fernandes et al., 2016). *S. typhimurium* invades the intestinal mucosa, multiplies within vesicles inside cells, and crosses epithelial 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 several decades (Armon et al., 1997; Lapidot et al., 2006; Yahya et al., 2017; Johari et al., 2023). Recent outbreaks and challenges in treating salmonellosis remain significant global health concerns (Mohamed et al., 2024; Oduoye et al., 2024; Pal et al., 2024). Despite advances in sanitation and food safety, Salmonella infections affect communities worldwide.

Transmembrane proteins confer specific properties to cellular membranes, including signal transduction and the transport of ions or small molecules. Some transmembrane proteins bind to hormone or neurotransmitter receptors, altering their structure and triggering specific cellular 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, transmembrane proteins are extensively researched for various applications, including sensors, screening, water purification, and energy harvesting (Ryu et al., 2019). Identifying transmembrane proteins in pathogenic microorganisms involves using bioinformatic tools and computational approaches to predict and analyze protein structures and functions. Sequence-based bioinformatics tools are employed to detect potential transmembrane domains within protein sequences, using algorithms such as 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 used to model the three-dimensional structures of the 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 identifying potential drug targets (Khan & Uddin, 2022). By comparing the genomes of drug-resistant strains with those of susceptible ones, bioinformatics helps detect genetic variations linked to resistance, thereby 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. Functional characterization and experimental validation of the identified transmembrane proteins in *S. typhimurium* are needed to determine their roles in pathogenesis and drug resistance. Investigating their involvement in specific biological activities and confirming their potential as drug targets could facilitate clinical studies to combat salmonellosis. Despite advancements in genomic and proteomic technologies, several proteins identified in *S. typhimurium* remain uncharacterized. Thus, the objective of the present study was to characterize the transmembrane proteins of *S. typhimurium* that can be used as drug and vaccine targets. This study addresses the knowledge gap surrounding *S. typhimurium* transmembrane proteins and pioneers identifying novel targets critical for pathogen survival and immune evasion. Such findings highlight the value of bioinformatics in accelerating target discovery and offer a robust foundation for developing precision therapeutics and vaccines to combat salmonellosis.

EXPERIMENTAL

Database archive

A total of one hundred fifty uncharacterized proteins from *Salmonella typhimurium* were randomly selected and obtained from the UniProtKB database. These proteins were subsequently downloaded in FASTA format for further analysis.

Bioinformatics tools and analysis

All downloaded protein sequences in FASTA format were analyzed using various bioinformatic tools. These sequences were input into the sequence query interface of several applications, including PSORTb V3.0.3, TMHMM, DEG, BLASTp, ProtParam, ScanProsite, and STRING 12.0, for comprehensive analysis and characterization. PSORTb is a bioinformatics tool to predict bacterial protein subcellular localization. It uses a combination of sequence features and sorting signals to predict whether a protein is in the cytoplasm, periplasm, inner membrane, outer membrane, or extracellular. This information is important for understanding protein function and interactions within bacterial cells. TMHMM (TransMembrane Hidden Markov Model) is used to predict transmembrane helices in proteins. It identifies whether a protein spans the membrane, how many transmembrane segments it contains, and where those segments are in the sequence. This is particularly useful for identifying membrane proteins, which play key roles in various biological processes.

DEG is a database that contains information on essential genes, those that are crucial for the survival of an organism. Researchers can use DEG to identify genes indispensable in various species, which can be potential targets for drug development or understanding fundamental biological processes. BLASTp is a widely used tool that compares a query protein sequence against a database of protein sequences to identify homologous sequences (Identity >30%; E-value $<1e^{06}$). It provides information about sequence similarity, allowing researchers to infer protein function, evolutionary relationships, and structural properties based on known proteins. ScanProsite is a tool used to search protein sequences for the presence of specific motifs, patterns, and domains defined in the Prosite database. Prosite contains information on protein families and domains, making this tool helpful for functional annotation of proteins and understanding their structural features.

STRING (Search Tool for the Retrieval of Interacting Genes/Proteins) is a database and web resource that predicts protein-protein interactions (PPI). Version 12.0 contains updated interactions based on experimental data, computational predictions, and public text mining. STRING helps researchers understand the functional networks and relationships between proteins, which is critical for studying pathways and biological systems.VaxiJen 2.0 server is a computational tool used for predicting the antigenicity of proteins based on their physicochemical properties, independent of sequence alignment. It categorizes proteins into antigens or non-antigens by analyzing molecular weight, pKa values, and mineral composition features.

RESULTS AND DISCUSSION

Figure 1 shows the classification of 150 uncharacterized proteins from *S. typhimurium* based on their biological pathways, molecular functions, subcellular localization, and identification as transmembrane proteins. Most 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.

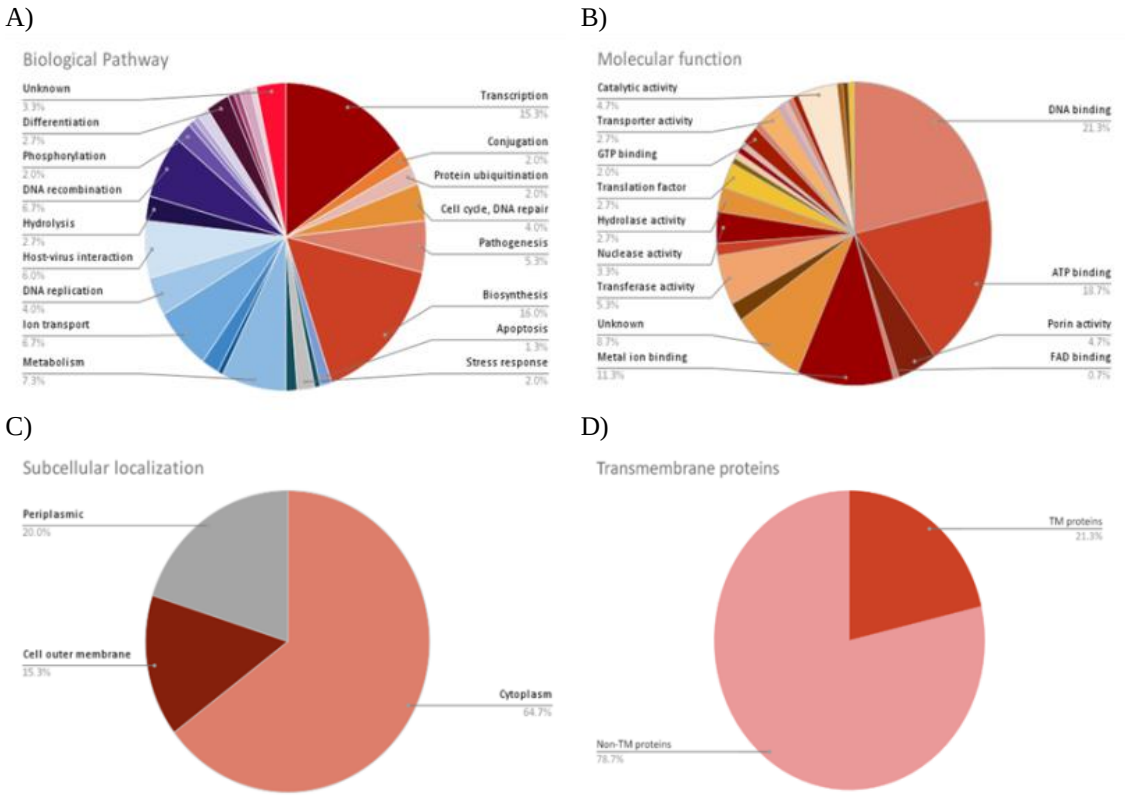


Fig. 1. Classification of uncharacterized protein of *S. typhimurium*, A) Biological pathway; B) Molecular function; C) Subcellular localization; D) Transmembrane proteins.

Table 1 lists 32 identified transmembrane proteins from *S. typhimurium*, along with details about the number of transmembrane segment (TMS) domains, their predicted biological pathways, and molecular functions. The number of TMS domains was predicted to vary across proteins. Biological pathways included DNA repair, cell cycle, protein transport, and energy metabolism. Molecular functions included ATP binding, GTP binding, ion transport, and various enzymatic activities. This information helps us understand how these proteins might interact with host cells or other cellular compartments during infection. Protein A0A2J0RKS1 showed the highest number of predicted transmembrane domains.

Table. 1. Identified transmembrane protein of *S. typhimurium.*

Accession	No. of TMS domain	Biological pathway	Molecular function
A0A2J0RKS1	9	Conjugation	Porin activity
A0A1P8DMP4	1	Unknown	ATP binding
A0A3Z7JEV9	1	DNA repair	DNA ligase activity
A0A2J0RFX8	1	Catabolic process	D-aminoacyl-tRNA deacylase activity
A0A2J0RDC1	1	Translocation	Unknown
A0A3T3ZZV4	1	tRNA processing	ATP binding
A0A3V6H1D5	1	Pathogenesis	Unknown
A0A0D6HCE5	2	Lipoprotein biosynthesis	phosphatidylglycerol-prolipoprotein diacylglyceryl transferase activity
A0A2J0RDS6	1	Host-virus interaction	Unknown
A0A3V7XF93	1	Carbohydrate metabolic process	Carbohydrate binding
A0A3V7X7Z5	2	Host-virus interaction	ATP binding
A0A5Z7LRR5	4	Proteolysis	Metal ion binding
A0A5Z7LRA6	1	Cell cycle	ATP binding
A0A0F7J9G5	1	DNA packaging	Metal ion binding
A0A0F7JGQ1	1	Cell adhesion	Protein-containing complex binding
A0A0F7JFI2	1	Differentiation	Zinc ion binding
A0A0F7JDX1	2	Establishment of competence for transformation	Unknown
A0A5Y2HQY6	1	Amino acid biosynthesis	ATP binding
A0A0F7J982	1	Protein biosynthesis	GTP binding
A0A6C8WQJ4	3	inorganic anion transport	Chloride channel activity
A0A735ZTN8	7	Transmembrane transport	Porin activity
A0A731QU75	2	Chemical synaptic transmission	G-protein coupled receptor activity [serotonin]
A0A607WTJ3	6	Phospholipid biosynthetic process	Transferase activity
A0A705WX69	1	Oxidative phosphorylation	Translocase
A0A707YZC5	1	Cytochrome complex assembly	heme transmembrane transporter activity
A0A706T8K4	2	Transmembrane transport	transmembrane transporter activity
A0A717VZE3	1	DNA replication	DNA binding
A0A736JL85	2	Folate biosynthesis	metal ion binding
A0A610AT56	3	Phospholipid biosynthetic process	Transferase activity
A0A705WZ57	1	Transmembrane transport	Transmembrane transporter activity
A0A701H8E2	4	Cytochrome c-type biogenesis	Heme binding
A0A7G2DIQ3	2	Viral tail assembly	Unknown

Figure 2 shows the physicochemical properties of identified transmembrane proteins of *S. typhimurium.* The color gradient ranges from green to red, with green representing longer sequences (up to ~1900 amino acids) and red indicating shorter sequences (~40 amino acids) (Figure 2A). Proteins with the longest sequences were clustered in the green area, while shorter proteins were in the red or yellow areas. The variety in protein sequence length may reflect the functional diversity of the proteins, where longer sequences may be associated with more complex structures or multifunctional roles. The isoelectric point (pI) values ranged from 4 (blue) to 11 (red) (Figure 2B). Proteins in the blue area have lower pI values (more acidic), while those in the red area have higher pI values (more basic). The pI is important for predicting protein solubility and behavior under different pH conditions, influencing their function in different environments. The color scale transitions from green (high molecular weight, ~200,000 Daltons) to red (low molecular weight, ~4,000 Daltons) (Figure 2C). Green shades indicate proteins with higher molecular weights.

In comparison, those with lower molecular weights are red. This can give insights into the complexity and size of the proteins, with larger proteins generally having more domains or functional elements. There is a distinct relationship among protein length, isoelectric point (pI), and molecular weight in *Salmonella typhimurium* proteins. Protein length (A) correlates directly with molecular weight (C), as longer proteins generally have higher molecular weights due to the increased number of amino acids contributing to the protein's mass. However, the isoelectric point (B) is independent of protein length and molecular weight, as it depends on the protein's net charge of the amino acid residues. Proteins with similar molecular weights can exhibit different pI values depending on their amino acid composition, particularly the balance of acidic and basic residues. The data suggests that molecular properties like pI are driven by sequence-specific characteristics rather than the physical size or weight of the protein.

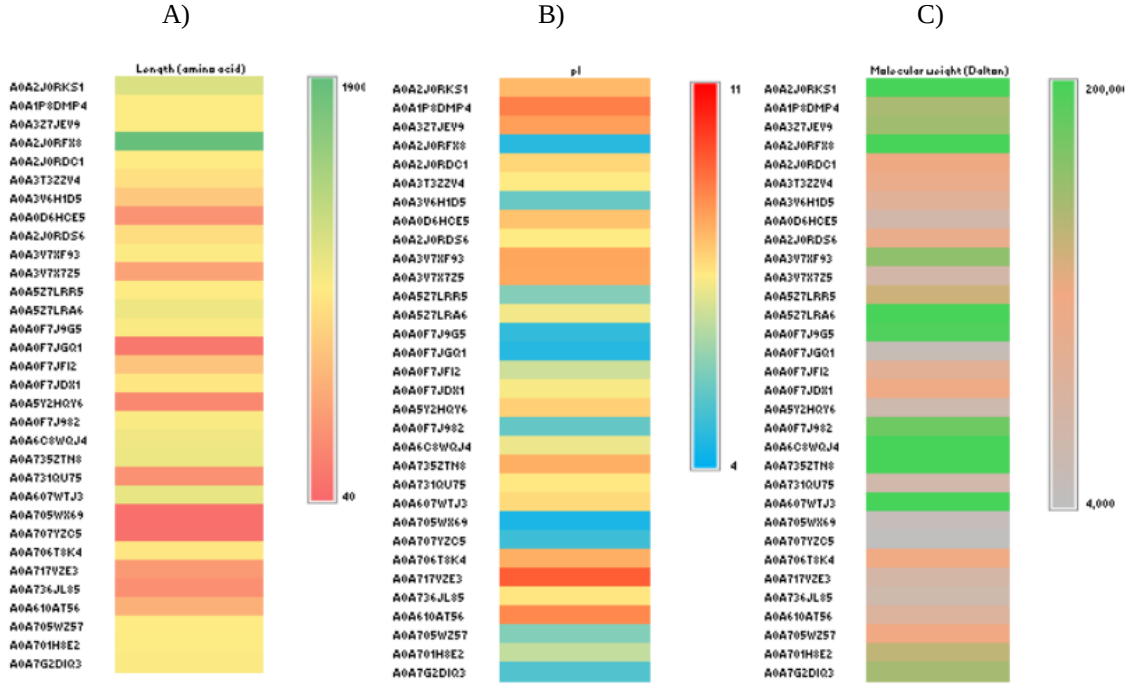


Fig. 2. Physicochemical properties of identified transmembrane proteins of *S. typhimurium.* A) protein length; B) Isoeletric point (pI); C) Molecular weight.

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. In addition, only protein A0A610AT56 was marked as a non-essential protein. Twenty-nine transmembrane proteins were predicted to be essential and non-host homologous. These proteins are potential drug targets because targeting them may inhibit the pathogen without harming the host. Researchers identify essential and non-host homologous proteins in drug design to develop more selective and effective therapies.

Table. 2. List of essential and non-host homologous transmembrane proteins of *S. typhimurium.* Identity < 30%. E-value >1e^{06}

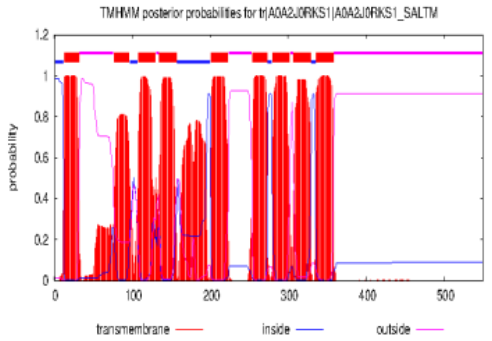
Protein	Human	Cattle	Sheep	Goat	Horses
A0A2J0RKS1	0	0	0	0	0
A0A1P8DMP4	0	0	0	0	0
A0A3Z7JEV9	0	0	0	0	0
A0A2J0RFX8	0	0	0	0	0
A0A2J0RDC1	0	0	0	0	0
A0A3T3ZZV4	0	0	0	0	0
A0A3V6H1D5	0	0	0	0	0
A0A0D6HCE5	0	0	0	0	0
A0A2J0RDS6	0	0	0	0	0
A0A3V7XF93	0	0	0	0	0
A0A3V7X7Z5	0	0	0	0	0
A0A5Z7LRR5	0	0	0	0	0
A0A5Z7LRA6	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 of homologues
(1) = presence of homologues
(*) = non-essential proteins

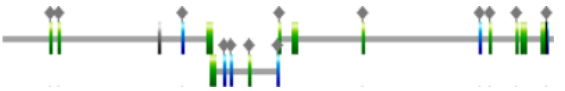
Figure 3 illustrates various analyses related to the transmembrane protein A0A2J0RKS1 of *S. typhimurium*, including its predicted transmembrane regions, post-translational modifications, and protein-protein interactions. The graph shows nine significant peaks above a certain threshold, representing 9 predicted transmembrane segments (TMSs) for A0A2J0RKS1. These regions suggest that the protein spans the membrane multiple times, a typical feature of proteins involved in transport, signaling, or cell communication. The linear depiction of the protein indicates potential post-translational modification sites, which include PKC phosphorylation site, N-glycosylation site, Myristoylation site, and CK2 phosphorylation site. Protein kinase C phosphorylation sites can regulate protein function by adding phosphate groups to serine/threonine residues, impacting signal transduction pathways. N-glycosylation sites add sugar moieties to asparagine residues, which is essential for protein folding, stability, and localization, especially for transmembrane proteins. Myristoylation refers to adding a lipid group, which facilitates membrane association and may be involved in protein-protein interactions. Casein kinase II phosphorylation may play roles in controlling cellular processes like DNA repair, cell cycle, and transcription regulation. These modifications suggest that A0A2J0RKS1 undergoes dynamic regulation

post-translation, likely influencing its role in the membrane, its interaction with other proteins, and its involvement in pathogenic processes. This network, generated using the STRING database, shows predicted interactions between A0A2J0RKS1 and other proteins based on various sources of evidence, such as experimental data, co-expression, and database annotations. Each node represents a protein; the edges (lines) connecting them indicate interactions or associations between these proteins. The colored edges correspond to different types of interaction evidence (e.g., experimental, curated databases, or predicted). Enriched biological processes and pathways in the protein-protein interaction network for A0A2J0RKS1 were not identified. However, A0A2J0RKS1 had 10 functional linkages with other proteins, making it crucial for the survival of *S. typhimurium*. A0A2J0RKS1 was also predicted to be antigenic. This analysis highlights A0A2J0RKS1 as a candidate for further experimental validation to confirm its role in bacterial virulence and drug resistance.

A)



B)



Post-translational modification sites:

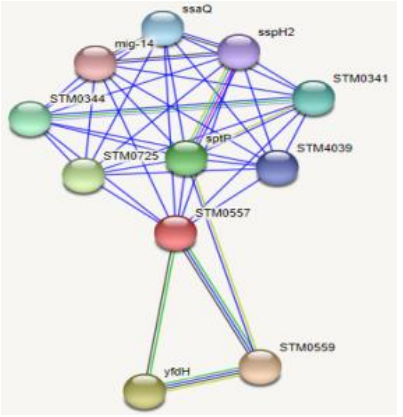
PKC_PHOSPHO_SITE Protein kinase C phosphorylation site;

ASN_GLYCOSYLATION N-glycosylation site;

MYRISTYL N-myristoylation site;

CK2_PHOSPHO_SITE Casein kinase II phosphorylation site

C)



D)

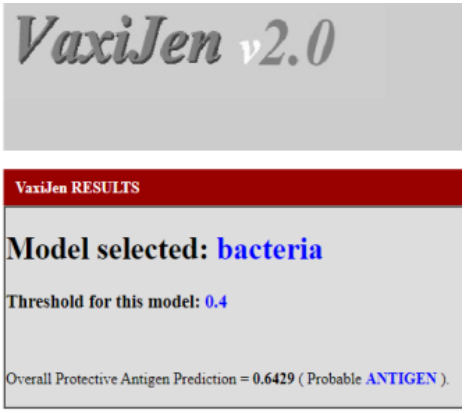


Fig. 3. TMS domains of A0A2J0RKS1; B) Functional motifs of A0A2J0RKS1; C) Protein-protein interaction network of A0A2J0RKS1 (STM0557); D) Antigenicity property.

The present study, *S. Typhimurium* proteins were analyzed using an *in silico* approach. This is crucial for understanding the pathogen's biology and developing targeted interventions, particularly given the challenges of working with live pathogens in a laboratory setting. Computational tools enable high-throughput analysis of protein structure, function, and interactions, providing valuable insights into

virulence factors, drug resistance mechanisms, and potential therapeutic targets. In silico methods, such as protein localization prediction, transmembrane domain identification, and protein-protein interaction networks, allow researchers to efficiently screen for essential, non-host homologous proteins that could serve as selective drug targets (Rashid et al. 2022; Nematiasgarabad et al. 2024; Zulkiply et al. 2024), thereby aiding the development of novel antimicrobial strategies. Additionally, these approaches are cost-effective and time-saving and can complement experimental data, making them an indispensable part of modern microbiology and bioinformatics research.

Transmembrane proteins play critical roles in the physiology and virulence of *S. typhimurium*, a Gram-negative bacterium responsible for various foodborne illnesses. These proteins span the bacterial cell membrane and are involved in essential functions like nutrient exchange, signal transduction, adhesion to host cells, and efflux of toxic compounds, including antibiotics (Sabnis et al. 2021). The outer membrane proteins (OMPs), such as porins, are essential, allowing selective permeability and contributing to antibiotic resistance by restricting the entry of harmful compounds (Safini et al. 2024). The present study explored the uncharacterized transmembrane proteins in *S. typhimurium.* Understanding these transmembrane proteins may provide insight into their functions and therapeutic potential (Attwood & Schiöth 2021; Saches et al. 2021). Transmembrane proteins are ideal targets for antibody-based therapies and vaccine development due to their accessibility on the cells' surface, making them easily recognized by antibodies. Transmembrane proteins typically have extracellular domains exposed on the cell surface, making them accessible to circulating antibodies. These extracellular regions can contain antigenic epitopes, specific sequences, or structural motifs antibodies can recognize and bind to. These epitopes often include loops, exposed helices, or glycosylation sites that protrude into the extracellular space. Antibodies can be highly specific, targeting distinct epitopes on the extracellular domain of transmembrane proteins. This specificity is critical for both therapeutic and vaccine development, as it ensures that the antibody only binds to the desired protein without cross-reacting with other proteins. The interaction between the antibody's variable region and the epitope is governed by the complementarity-determining regions (CDRs), which are the portions of the antibody responsible for recognizing and binding to antigens. Once an antibody binds to a transmembrane protein, it can elicit various immune responses. For vaccine development, this binding may neutralize the protein's function, mark the cell for destruction by immune cells, or block important receptor-ligand interactions essential for the pathogen's survival.

As shown by the present study, the quantity of TMS domains was predicted to differ across uncharacterized *S. typhimurium* proteins. A higher number of transmembrane domains may contribute to the stability of the protein within the membrane, but this stability does not necessarily enhance its potential as a vaccine target. The antigenicity, or the ability of the extracellular regions to be recognized by the immune system, is key. Therefore, even a protein with a single transmembrane domain could be an excellent vaccine target if it has a large, antigenic extracellular domain. The pI values of the transmembrane proteins showed a wide range (pI 4–11), but the functional consequences of this variation remain unexplored. Transmembrane proteins function in environments of varying pH. A more thorough analysis should explore whether there is a pattern or bias in pI values among specific subgroups of transmembrane proteins, such as outer and inner membrane proteins. Proteins with extreme pI values may be suitable for therapeutic targeting due to solubility or stability issues, especially in neutral or physiological pH environments. The present finding effectively describes the sequence length, pI, and molecular weight trends individually. However, a deeper evaluation would benefit from discussing potential correlations between these properties. Exploring these connections would provide a more integrated view of the protein set and could highlight proteins with unique combinations of properties that might be functionally significant.

Essential proteins are necessary for the bacterium's survival, replication, and virulence. When absent in host organisms, these proteins are excellent candidates for antimicrobial drugs because targeting them would likely cause minimal side effects to the host. The absence of homologous proteins in humans or other hosts could prevent off-target effects, reducing toxicity and increasing the therapeutic window of potential

drugs. Most transmembrane proteins of *S. typhimurium* identified in the present study 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). Experimental validation through *in vitro* assays, such as ELISA for antigenicity, mutational studies for essentiality, and structural bioinformatics to confirm druggability, can provide robust evidence for their candidacy as therapeutic or vaccine targets. Computational approaches have been used to analyze bacterial proteomes, identifying essential proteins non-homologous to human proteins and involved in unique metabolic pathways (Rahman et al., 2014; Rahman et al., 2019). These studies have revealed promising targets in pathogens such as *Bacillus anthracis* and *Salmonella enterica*.

Protein A0A2J0RKS1, with the most transmembrane domains, was predicted to have porin activity and numerous functional linkages, establishing it as a significant hub protein in *S. typhimurium*. Porin proteins play crucial roles in *S. typhimurium* pathogenesis. For example, OmpA contributes to intracellular virulence by maintaining *Salmonella*-containing vacuole (SCV) stability and protecting against nitrosative stress in macrophages (Roy Chowdhury et al., 2022). OmpA deletion increases outer membrane porosity and susceptibility to nitrosative stress, while its presence helps regulate other porin expressions. OmpD, another porin, is downregulated during infection, and its deletion enhances bacterial survival and replication in macrophages and mice, possibly by limiting host reactive oxygen species response (Ipinza et al., 2014). These studies highlight the complex and multifaceted roles of porin proteins in *S. typhimurium*'s interactions with host cells and immune responses, contributing to its pathogenicity and survival within the host. According to Zawawi et al. (2020), porins are the common indicators for the immediate response of microbial cells towards a wide range of antimicrobial agents. Meanwhile, identifying essential hub proteins in pathogenic microorganisms via protein-protein interaction networks has been reported in several studies (Yahya et al., 2017; Rashid et al., 2022; Isa et al., 2022; Zulkiply et al., 2022; Bajire et al. 2023; Nithya et al., 2023). A study on methicillin-resistant *Staphylococcus aureus* (MRSA) revealed that current antimicrobial targets often occupy peripheral positions in the protein-protein interaction networks, suggesting that targeting hub proteins could be a more effective strategy for drug development (Cherkasov et al., 2011).

Several identified transmembrane proteins of *S. typhimurium* identified herein had amino acid lengths shorter than 100 residues. A minimum threshold of 100 amino acids should be considered when annotating protein sequences because proteins shorter than 100 may be incomplete or represent small peptides lacking well-defined structure or function. Additionally, at least 100 amino acid sequences are generally more likely to have a stable, functional 3D structure and contain sufficient evolutionary and functional information for reliable bioinformatics analyses. Many functional protein domains are larger than 100 amino acids. By filtering out shorter sequences, researchers could reduce the chances of including sequences that may not contain a fully functional domain, leading to more meaningful analyses. Very short sequences are more prone to low-complexity regions, which are repetitive and less informative for functional or comparative analyses. However, research has explored the potential of low molecular weight proteins as vaccine targets against various pathogens. Studies on *Ehrlichia ruminantium* identified five proteins smaller than 20 kDa that induced immune responses and partially protected sheep (Sebatjane et al., 2010). This finding highlights the promise of low molecular weight and membrane proteins as vaccine candidates against various pathogens.

The present study emphasizes characterizing transmembrane proteins of *S. typhimurium*, detailing a systematic approach to analyze 150 uncharacterized proteins and identifying protein A0A2J0RKS1 as a promising candidate. In contrast, Iftikhar et al. (2020) provide broader background information about the pathogenicity and mortality rates of *S. typhimurium*, focusing on a subtractive proteome mining approach to identify 36 essential genes and 15 pathways as potential targets. Unlike the present study, they highlight outer membrane proteins as significant targets and anticipate virtual screening for clinical evaluation.

CONCLUSION

We demonstrated that 32 uncharacterized transmembrane proteins of *S. typhimurium* hold promise as drug targets. They were predicted to be essential and non-host homologous. Ongoing analysis of these transmembrane proteins is crucial to aid in developing effective drugs targeting Salmonella infection. By leveraging advanced proteomic tools to identify and characterize transmembrane proteins, this study paves the way for developing novel therapeutic interventions. Future research should validate these findings through experimental models, optimize candidate proteins for vaccine and drug design, and integrate these approaches with global vaccination programs and antimicrobial resistance strategies.

ACKNOWLEDGMENTS

The authors would like to acknowledge the support of the Faculty of Applied Sciences, Universiti Teknologi MARA, Shah Alam, Selangor, Malaysia, for providing the facilities.

AUTHOR'S CONTRIBUTION

Izzati Nasuha Mohd Samsidi, Nawal Zulkiply, and Anati Abd Rashid Syaida conducted the research and wrote and revised the article. Mohd Fakharul Zaman Raja Yahya conceptualized the central research idea, provided the theoretical framework, designed the research, supervised the research progress, anchored the review, and approved the article submission.

CONFLICT OF INTEREST STATEMENT

The authors affirm that there are no competing interests regarding the publication of this paper.

REFERENCES

Armon, R., Starosvetzky, J., Arbel, T., & Green, M. (1997). Survival of *Legionella pneumophila* and *Salmonella typhimurium* in biofilm systems. *Water Science and Technology, 35*(11-12), 293-300.

Attwood, M. M., & Schiöth, H. B. (2021). Characterization of five transmembrane proteins: Focusing on the Tweety, sideroflexin, and yip1 domain families. *Frontiers in Cell and Developmental Biology, 9*, 708754.

Bajire, S. K., Ghate, S. D., Shetty, S., Banerjee, S., Rao, R. S. P., Shetty, V., & Shastry, R. P. (2023). Unveiling the role of hub proteins in controlling quorum sensing regulated virulence through analogs in *Pseudomonas aeruginosa* PAO1: A functional protein-protein network biology approach. *Biochemical and Biophysical Research Communications, 660*, 13-20.

Cherkasov, A., Hsing, M., Zoraghi, R., Foster, L. J., See, R. H., Stoynov, N., Jiang, J., Kaur, S., Lian, T., Jackson, L., Gong, H., Swayze, R., Amandoron, E., Hormozdiari, F., Dao, P., Sahinalp, C., Santos-Filho, O., Axerion-Cilies, P., Byler, K., McMaster, W. R., Brunham, R. C., Finlay, B. B., & Reiner, N. E. (2011). Mapping the protein interaction network in methicillin-resistant *Staphylococcus aureus*. *Journal of Proteome Research, 10*(3), 1139-1150.

Chowdhury, A. R., Sah, S., Varshney, U., & Chakravortty, D. (2022). *Salmonella Typhimurium* outer membrane protein A (OmpA) renders protection from nitrosative stress of macrophages by maintaining the stability of bacterial outer membrane. *PLoS Pathogens, 18*(8), e1010708.

Fernandes, L., Centeno, M. M., Couto, N., Nunes, T., Almeida, V., Alban, L., & Pomba, C. (2016). Longitudinal characterization of monophasic *Salmonella typhimurium* throughout the pig's life

cycle. *Veterinary Microbiology, 192*, 231–237.
Hapfelmeier, S., & Hardt, W.-D. (2005). A mouse model for *Salmonella typhimurium*-induced enterocolitis. *Trends in Microbiology, 13*(10), 497–503.
Ipinza, F., Collao, B., Monsalva, D., Bustamante, V. H., Luraschi, R., Alegría-Arcos, M., Almonacid, D. E., Aguayo, D., Calderón, I. L., Gil, F., Santiviago, C. A., Morales, E. H., Calva, E., & Saavedra, C. P. (2014). Participation of the *Salmonella* OmpD porin in the infection of RAW264. 7 macrophages and BALB/c mice. *PloS one*, *9*(10), e111062.
Iftikhar, R., Rizwan, M., Khan, S., Mehmood, A., & Munir, A. (2020). Subtractive Proteome Mining Approach towards Unique Putative Drug Targets Identification for Salmonella typhimurium. *Infectious Disorders-Drug Targets*, *20*(6), 884-892.
Isa, S. F. M., Abdul Hamid, U. M., & Zaman Raja Yahya, M. F. (2022). Treatment with the combined antimicrobials triggers proteomic changes in *P. aeruginosa-C. albicans* polyspecies biofilms. *ScienceAsia*, *48*(2), 215-222.
Jalal, K., Khan, K., Hassam, M., Abbas, M. N., Uddin, R., Khusro, A., Sahibzada, M. U. K., & Gajdács, M. (2021). Identification of a novel therapeutic target against XDR *Salmonella* Typhi H58 using genomics driven approach followed up by natural products virtual screening. *Microorganisms, 9*(12), 2512.
Johari, N. A., Aazmi, M. S., & Yahya, M. F. Z. R. (2023). FTIR spectroscopic study of inhibition of chloroxylenol-based disinfectant against *Salmonella enterica* serovar Thyphimurium Biofilm. *Malaysian Applied Biology, 52*(2), 97-107.
Khan, K., & Uddin, R. (2022). Integrated bioinformatics based subtractive genomics approach to decipher the therapeutic function of hypothetical proteins from *Salmonella typhi* XDR H-58 strain. *Biotechnology Letters, 44*, 279-298.
Lapidot, A., Romling, U., & Yaron, S. (2006). Biofilm formation and the survival of *Salmonella typhimurium* on parsley. *International Journal of Food Microbiology, 109*(3), 229-233.
Mohammed, B. T. (2022). Identification and bioinformatic analysis of invA gene of *Salmonella* in free range chicken. *Brazilian Journal of Biology, 84*, e263363.
Mohamed, M. Y. I., Habib, I., & Khalifa, H. O. (2024). Salmonella in the food chain within the Gulf Cooperation Council countries. *AIMS microbiology*, *10*(3), 468.
Nithya, C., Kiran, M., & Nagarajaram, H. A. (2023). Dissection of hubs and bottlenecks in a protein-protein interaction network. *Computational Biology and Chemistry, 102*, 107802.
Nematiasgarabad, P., Hashim, N. A. N., & Yahya, M. F. Z. R. (2024). In silico elucidation of protein-protein interaction network in fish pathogen Flavobacterium Columnare. *Malaysian Applied Biology*, *53*(3), 137-146.
Nogueira, W. G., Jaiswal, A. K., Tiwari, S., Ramos, R. T., Ghosh, P., Barh, D., Azevedo, V., & Soares, S. C. (2021). Computational identification of putative common genomic drug and vaccine targets in *Mycoplasma genitalium*. *Genomics, 113*(4), 2730-2743.
Oduoye, M. O., Akilimali, A., Karim, K. A., Moradeyo, A. A., Abdullahi, Z. Z., Muzata, D., ... & Isiko, I. (2024). Diseases Transmitted to Humans through Foodborne Microbes in the Global South. In *Food Safety and Quality in the Global South* (pp. 561-597). Singapore: Springer Nature Singapore.
Okoro, C. K., Barquist, L., Connor, T. R., Harris, S. R., Clare, S., Stevens, M. P., Arends, M. J., Hale, C., Kane, L., Pickard, D. J., Hill, J., Harcourt, K., Parkhill, J., Dougan, G., & Kingsley, R. A. (2015). Signatures of adaptation in human invasive *Salmonella* Typhimurium ST313 populations from Sub-Saharan Africa. *PLOS Neglected Tropical Diseases, 9*(3), e0003611.
Othman, N. A., & Yahya, M. F. Z. R. (2019). *In silico* analysis of essential gene and non-homologous proteins in *Salmonella typhimurium* biofilm. *Journal of Physics: Conference Series, 1349*, 012133.
Pal, M., Ragasa, T., Rebuma, T., & Zende, R. (2024). Salmonellosis Remains the Hidden Menace in Our Global Food Supply: A Comprehensive Review. *American Journal of Medical and Biological Research*, *12*(1), 1-12.
Rahman, M. A., Noore, M. S., Hasan, M. A., Ullah, M. R., Rahman, M. H., Hossain, M. A., ... & Islam, M. S. (2014). Identification of potential drug targets by subtractive genome analysis of Bacillus anthracis A0248: An in silico approach. *Computational biology and chemistry*, *52*, 66-72.
Rahman, N., Muhammad, I., Nayab, G. E., Khan, H., Filosa, R., Xiao, J., & Hassan, S. T. (2019). In-silico subtractive proteomic analysis approach for therapeutic targets in MDR Salmonella enterica subsp. enterica serovar Typhi str. CT18. *Current Topics in Medicinal Chemistry*, *19*(29), 2708-2717.
Rashid, S. A. R., Yaacob, M. F., Raihanah, N., Anuar, T., Johari, N., Kamaruzzaman, A. N. A., Yunus, N.

M., & Yahya, M. F. Z. R. (2022). A combination of *in silico* subtractive and reverse vaccinology approaches reveals potential vaccine targets in *Corynebacterium pseudotuberculosis. Journal of Sustainability Science and Management, 17*(1), 99-109.

Ryu, H., Fuwad, A., Yoon, S., Jang, H., Lee, J. C., Kim, S. M., & Jeon, T. (2019). Biomimetic membranes with transmembrane proteins: State-of-the-art in transmembrane protein applications. *International Journal of Molecular Sciences, 20*(6), 1437.

Sabnis, A., Hagart, K. L. H., Klöckner, A., Becce, M., Evans, L. E., Furniss, R. C. D., Mavridou, D. A. I., Murphy, R., Stevens, M. M., Davies, J. C., Larrouy-Maumus, G. J., Clarke, T. B., & Edwards, A. M. (2021). Colistin kills bacteria by targeting lipopolysaccharide in the cytoplasmic membrane. Microbiology and Infectious Disease, 10, e65836.

Safini, I. N. M., Zakaria, N. F. S., Saad, M. I. H., Yahya, M. F. Z., & Jamil, N. M. (2024). Understanding Bacterial Persistence under Antibiotic Pressure: A Review. *Science Letters, 18*(2), 56-69.

Sanches, R. C., Tiwari, S., Ferreira, L. C., Oliveira, F. M., Lopes, M. D., Passos, M. J., Maia, E. H. B., Taranti, A. G., Kato, R., Azavedo, V. A. C., & Lopes, D. O. (2021). Immunoinformatics design of multi-epitope peptide-based vaccine against *Schistosoma mansoni* using transmembrane proteins as a target. *Frontiers in Immunology, 12,* 621706.

Sebatjane, S. I., Pretorius, A., Liebenberg, J., Steyn, H., & Van Kleef, M. (2010). In vitro and in vivo evaluation of five low molecular weight proteins of Ehrlichia ruminantium as potential vaccine components. *Veterinary Immunology and Immunopathology, 137*(3-4), 217-225.

Yahya, M. F. Z. R., Hamid, U. M. A., Norfatimah, M. Y., & Kambol, R. (2014). *In silico* analysis of essential tricarboxylic acid cycle enzymes from biofilm-forming bacteria. *Trends in Bioinformatics, 7*(1), 19–26.

Yahya, M. F., Alias, Z., & Karsani, S. A. (2017). Subtractive protein profiling of *Salmonella typhimurium* biofilm treated with DMSO. *The Protein Journal, 36*(4), 286–298.

Zawawi, W. M. A. W. M., Ibrahim, M. S. A., Rahmad, N., Hamid, U. M. A., & Yahya, M. F. Z. R. (2020). Proteomic analysis of Pseudomonas aeruginosa biofilm treated with Chromolaena odorata extracts. *Malaysian Journal of Microbiology, 16*(2), 124-133.

Zhang, R., Ou, H., & Zhang, C. (2004). DEG: A database of essential genes. *Nucleic Acids Research, 32,* D271-D272.

Zulkiply, N., Ramli, M. E., & Yahya, M. F. Z. R. (2022). *In silico* identification of antigenic proteins in *Staphylococcus aureus*. *Journal of Sustainability Science and Management, 17*(2), 18-26.

Zulkiply, N., Yunus, N. M., Idris, H., Noor, W. S. A., W. M., Karsani, S. A., Yahya, M. F. Z. R. (2024). *In silico* and MS/MS-based approaches to investigate protein-protein interaction networks in Staphylococcus aureus biofilm. *Malaysian Journal of Analytical Science*, 28(4), 939-955.