Cloning and Bioinformatics Analysis of *trxB* Gene in *Vibrio alginolyticus* HY9901

Yuyan HE[1,2#], Xuelian LIN[1,2#], Zhihang CHEN[1,2], Xiaoxin WEN[1,2], Jianyi WEI[3], Huanying PANG[1,2*]

1. Fisheries College, Guangdong Ocean University, Zhanjiang 524025, China; 2. Guangdong Provincial Key Laboratory of Aquatic Animal Disease Control and Healthy Culture & Key Laboratory of Control for Diseases of Aquatic Economic Animals of Guangdong Higher Education Institutes, Zhanjiang 524025, China; 3. Guangxi Beihai Nanjiang Aquatic Products Co., Ltd, Beihai 536000, China

Abstract [**Objectives**] This study was conducted to investigate the functional characteristics of the *trxB* gene in *Vibrio alginolyticus*. [**Methods**] A pair of specific primers was designed based on the *trxB* gene sequence of *V. alginolyticus* for PCR cloning of its full-length sequence. Systematic bioinformatics analyses were conducted to predict the physicochemical properties, secondary structure, and tertiary structure of the encoded protein. [**Results**] The *trxB* gene is 960 bp in length, encoding 319 amino acid residues. The deduced protein has a predicted molecular weight of 34.32 kDa and an isoelectric point (pI) of 4.77. Analysis of the amino acid sequence revealed a distinct signal peptide cleavage site at the N-terminus, with no transmembrane domains. The functional sites are as follows: 1 N-glycosylation site, 1 cAMP- and cGMP-dependent protein kinase phosphorylation site, 4 protein kinase C phosphorylation sites, 7 casein kinase II phosphorylation sites, 1 tyrosine kinase phosphorylation site, 11 N-myristoylation sites, 1 prenyl group binding site, 3 microbody C-terminal targeting signal sites, and 1 xanthine nucleotide-disulfide oxidoreductase class II active site. Subcellular localization prediction indicated the highest probability (44.4%) for endoplasmic reticulum localization. The TrxB amino acid sequence of *V. alginolyticus* shares 97.2% -98.4% homology with other *Vibrio* species, and they were clustered within the same subgroup. Secondary structure prediction showed proportions of random coils (31.97%), alpha-helices (31.66%), extended strands (25.08%), and beta turns (11.29%). The tertiary structure model exhibited 88.68% similarity to template 5vt3.1.A. [**Conclusions**] This study elucidated the characterization of the TrxB protein in *V. alginolyticus*, laying a theoretical foundation for the development of outer membrane protein subunit vaccines against this pathogen.

Key words *Vibrio alginolyticus*; Gene cloning; *trxB* gene; Bioinformatics analysis

DOI:10.19759/j.cnki.2164 -4993.2025.02.001

Vibrio alginolyticus, belonging to *Vibrio* in Vibrionaceae, is a Gram-negative, anaerobic short rod-shaped bacterium. This non-capsulated and non-spore-forming pathogen possesses both polar and lateral flagella, serving as one of the major opportunistic pathogens causing vibriosis in marine aquaculture species[1]. As a zoonotic pathogen, *V. alginolyticus* threatens human health through direct invasion or consumption of contaminated seafood, potentially causing diarrhea, septicemia, otitis media, and food-borne illnesses[2-3]. Therefore, in-depth investigation of this bacterium's pathogenic mechanisms is of significant economic value and social importance to preventing and controlling its associated infections and disease transmission. Current research indicates that the pathogenicity of *V. alginolyticus* is closely associated with various virulence factors, including well-studied extracellular proteases, biofilms, adhesins (encompassing outer membrane proteins and lipopolysaccharides), and iron acquisition systems[4], while few studies have been conducted on its thioredoxin (Trx) system.

The Trx system consists of Trx, thioredoxin reductase (TrxR), and reduced nicotinamide adenine dinucleotide phosphate (NADPH), playing crucial roles in cellular proliferation, survival regulation, and redox balance and antioxidant defense in organisms. In addition to sustaining redox homeostasis and regulating signal transduction, this system participates in redox reactions, nucleic acid metabolism, cellular growth and functional regulation, and has even been closely linked to tumorigenesis[5-7]. Furthermore, the Trx system can activate NF-κB and its associated signaling pathways[8-9]. Studies have revealed that *E. coli* strain AD494, carrying a mutation in the thioredoxin reductase (*trxB*) gene, promotes intracellular disulfide bond formation, thereby facilitating proper protein folding and ultimately generating biologically active functional proteins[10]. These findings fully confirm the important position of Trx system in organisms.

In this study, in order to investigate the function of the *trxB* gene in *V. alginolyticus*, systematic bioinformatics analysis was conducted on the cloned gene sequence, including its physicochemical properties, secondary structure, and tertiary structure, and the characterization features of the TrxB protein in *V. alginolyticus* were preliminarily elucidated, laying a theoretical foundation for subsequent development of outer membrane protein subunit vaccines against *V. alginolyticus*[11].

Materials and Methods

Materials

Bacterial strain Virulent *V. alginolyticus* strain HY9901 was

Received: January 5, 2025 Accepted: March 9, 2025

Supported by Natural Science Foundation of Guangdong Province (2025A1515011061); Outstanding Graduate Entering Laboratory Project of College of Fisheries, Guangdong Ocean University; Undergraduate Innovation and Entrepreneurship Training Program of Guangdong Ocean University (CXXL2024007); Undergraduate Innovation Team of Guangdong Ocean University (CCTD201802).

Yuyan He (2004 -), female, P. R. China, major: aquatic animal medicine. Xuelian LIN (2002 -), female, P. R. China, major: aquatic animal medicine. #These authors contributed to the work equally.

*Corresponding author. Huanying PANG, associate professor, PhD, devoted to research about aquatic veterinary medicine.

isolated from diseased *Lutjanus sanguineus* in coastal waters of Zhanjiang City, Guangdong Province and preserved in our laboratory.

Main reagents ExTaq DNA polymerase was purchased from Takara. Bacterial genomic DNA extraction kit and DNA gel recovery kit were obtained from Tiangen. All other reagents were analytically pure, from domestic or international suppliers. PCR primer synthesis and sequencing were performed by Sangon Biotech (Shanghai). Ampicillin (Ap) was applied at a concentration of 100 μg/ml.

Methods

Extraction of total DNA from *V. alginolyticus* HY9901 The HY9901 strain was streaked onto TSA plates, and single colonies were picked and inoculated into TSB (5% NaCl) medium, followed by shaking culture at 28 ℃ for over 12 h. Bacteria were collected by centrifugation at 10 000 rpm for 1 min, and genomic DNA was extracted according to the kit instructions and stored at −20 ℃ for further use.

Cloning of *trxB* gene A pair of primers targeting the *trxB* gene of *V. alginolyticus* was designed: forward primer P1 (ATGAGCGACGTAAAACACTGTAAAT) and reverse primer P2 (TTATTTGTCACCAAGTGAGTCTAAG). PCR amplification was performed using the extracted HY9901 genomic DNA as template under following conditions: initial denaturation at 94 ℃ for 5 min, 35 cycles of 94 ℃ for 30 s, 53 ℃ for 30 s and 72 ℃ for 40 s, and final extension at 72 ℃ for 10 min. The PCR product was analyzed by 1.5% agarose gel electrophoresis, purified using a DNA gel extraction kit, and cloned into the pMD18-T vector to construct a recombinant plasmid, designated pMD-*trxB*.

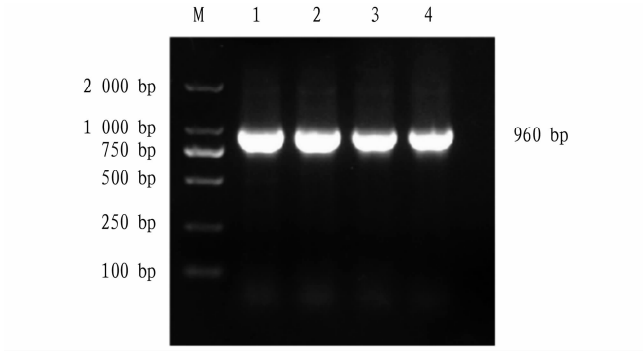
Bioinformatics analysis of *trxB* from *V. alginolyticus* HY9901

Bioinformatics analysis was performed following the method described by Liang *et al.* [12] using bioinformatics websites. Sequence homology and similarity were analyzed using NCBI BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Amino acid homology alignment was conducted using DNAMAN Version 6.0 (Lynnon Biosoft). ORF Finder (http://www.ncbi.nlm.nih.gov/gorf/gorf.html) and ExPASy Proteomics Server (http://ca.expasy.org) were employed to deduce the amino acid sequence, identify open reading frames (ORFs), and predict molecular weight (Mw) and theoretical isoelectric point (pI). Signal peptide sequences were predicted using SignalP 5.0 Server (https://services.healthtech.dtu.dk/service.php? SignalP-5.0). Transmembrane domains were predicted with TMHMM Server 2.0 (http://www.cbs.dtu.dk/services/TMHMM). Functional sites in the amino acid sequence were predicted using SoftBerry-Psite (http://linux1.softberry.com/berry.phtml? topic = psite&group = programs&subgroup = proloc). Protein structural domains were analyzed using InterProScan Sequence Search (http://www.ebi.ac.uk/Tools/InterProScan). Subcellular localization was predicted with PSORT II Prediction (http://psort.hgc.jp/form2.html). A phylogenetic tree was constructed using the neighbor-joining method with Clustal 2.0 and MEGA 5.0 software. Modeling was performed using SWISS-MODEL (http://www.swissmodel.expasy.org/) on the ExPASy server, and the resulting model was analyzed using 3D structural analysis software PyMOL Viewer.

Results and Analysis

Amplification of *trxB* gene

A specific band of approximately 960 bp was successfully obtained by PCR (Fig. 1). The gene encodes 319 amino acids.



M: DL2000DNA molecular weight standard; Lane 1 − 4: *trxB* PCR products.

Fig. 1 Amplification of *trxB* gene

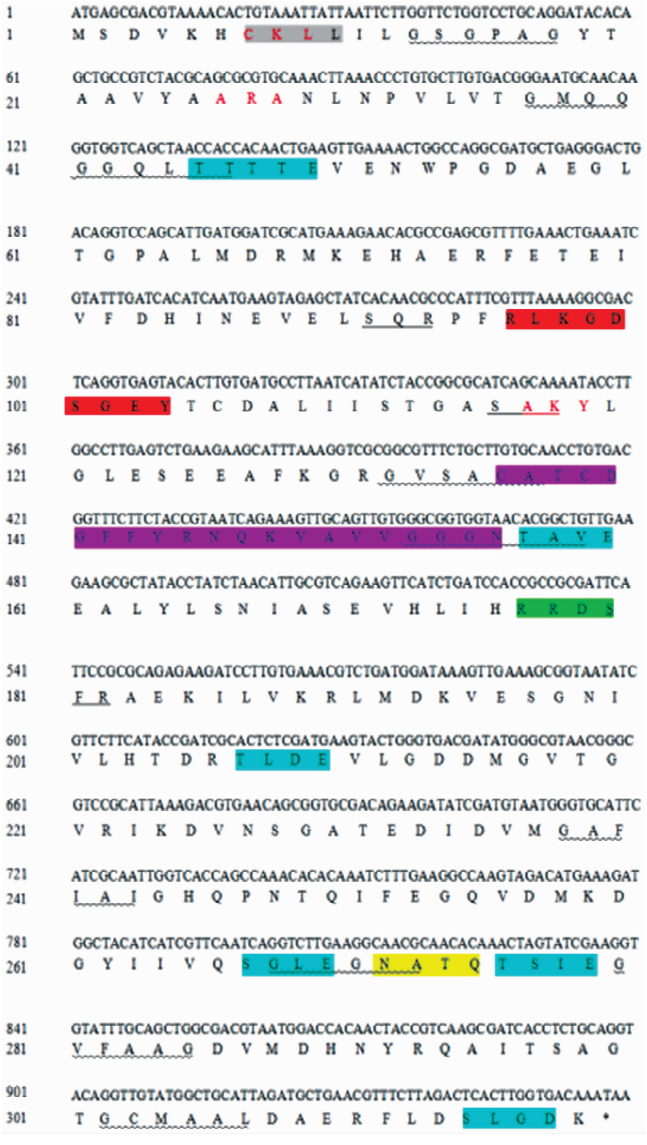
Physicochemical properties of *trxB*

Analysis of the *V. alginolyticus* HY9901 TrxB protein using ExPASy software revealed a total of 4 769 atoms, with a molecular formula of $C_{1\,489}H_{2\,365}N_{417}O_{483}S_{15}$. The theoretical molecular weight is 34 317.56 Da, and the theoretical pI is 4.77. The instability index is 31.29, and the aliphatic index is 85.02. The average of hydropathicity (GRAVY) is −0.166, indicating the protein is hydrophobic overall. The protein contains no selenocysteine (Sec) or pyrrolysine (Pyl). Assuming all Cys residues are reduced, the molar extinction coefficient at 280 nm is 17 420 M^{-1} cm^{-1}. The total number of acidic amino acids (Asp + Glu) is 47, while the number of basic amino acids (Arg + Lys) is 28. The N-terminus is methionine (Met). The half-life was predicted to be >20 h in yeast, >10 h in *Escherichia coli*, and 30 h in mammalian reticulocytes (*in vitro*).

Sequence analysis of *trxB*

The SignalP 5.0 Server predicted a clear signal peptide cleavage site in the N-terminal region of the TrxB amino acid sequence, indicating the presence of a signal peptide. TMHMM Server 2.0 analysis revealed no transmembrane domains in the protein. Using SoftBerry-Psite, following functional sites were predicted: 1 N-glycosylation site (272 − 275 aa), 1 cAMP- and cGMP-dependent protein kinase phosphorylation site (177 − 180 aa), 4 protein kinase C phosphorylation sites (91 − 93 aa, 116 − 118 aa, 180 − 182 aa, 204 − 206 aa), 7 casein kinase II phosphorylation sites (46 − 49 aa, 48 − 51 aa, 157 − 160 aa, 207 − 210 aa, 267 − 270 aa, 276 − 279 aa, 315 − 318 aa), 1 tyrosine kinase phosphorylation site (96 − 104 aa), 11 N − myristoylation sites (13 − 18 aa, 37 − 42 aa, 41 − 46 aa, 42 − 47 aa, 132 − 137 aa, 153 − 158 aa, 154 − 159 aa, 238 − 243 aa, 268 − 273 aa, 280 − 285 aa, 302 − 307 aa), 1 prenyl group binding site (7 − 10 aa),

3 microbody C-terminal targeting signals (7 – 9 aa, 26 – 28 aa, 117 – 119 aa), and 1 xanthine nucleotide-disulfide oxidoreductase class II active site (136 – 156 aa) (Fig. 2). Subcellular localization prediction suggested that *trxB* is most likely localized in the endoplasmic reticulum (44.4%), followed by the plasma membrane (33.3%), with lower probabilities in the Golgi apparatus (8.7%) and vacuole (8.7%).



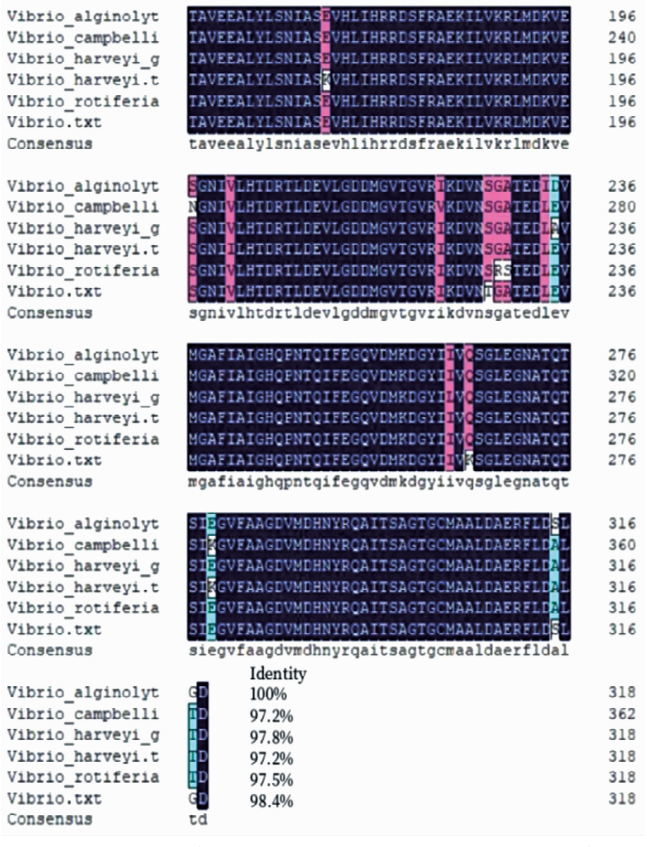
The terminator is represented by *; the yellow part represents the N-glycosylation site; the green part represents cAMP and cgmp dependent protein kinase phosphorylation sites; the black underline represents the protein kinase C phosphorylation site; the blue part represents the casein kinase II phosphorylation site; the red part represents the tyrosine kinase phosphorylation site; the black bottom line represents the n-terminal myristoylation site; Gray represents isopentenyl binding sites; the red part represents the micro C-terminal target signal site; and the purple portion represents the xanthine nucleotide-disulfide redoxreductase class II active site.

Fig. 2 Nucleotides of *trxB* gene and its encoded amino acid sequence

Homology and evolutionary analysis of TrxB

BLAST analysis revealed that the *trxB* gene of *V. alginolyticus* shares high homology with *trxB* from other *Vibrio* species, exhibiting up to 98.4% amino acid sequence homology. Multi-sequence similarity comparison demonstrated that *trxB* is highly conserved among *Vibrio* species (Fig. 3).

The deduced TrxB amino acid sequence and other microorganisms were used to construct a phylogenetic tree by the Neighbor-joining method in MEGA 5.0 software. The results showed that the *trxB* protein of *V. alginolyticus* were clearly clustered within the same subgroup as other *Vibrio* species, indicating a close phylogenetic relationship among them (Fig. 4).



V. alginolyticus (WP_258453976.1); *Vibrio campbellii* (WP_047479081.1); *Vibrio harveyi group* (WP_182026273.1); *Vibrio harveyi* (WP_222325531.1); *Vibrio rotiferianus* (WP_143692652.1); *Vibrio* (WP_047008641.1).

Fig. 3 Comparison of derivable amino acid sequence homology of *trxB* gene

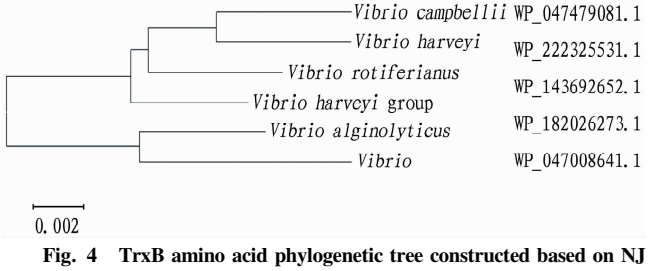


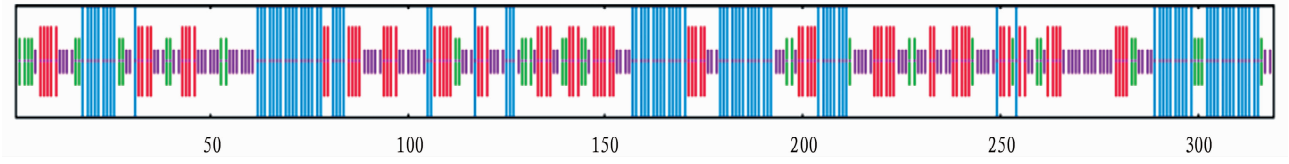
Fig. 4 TrxB amino acid phylogenetic tree constructed based on NJ method

Prediction of functional domains and secondary and tertiary structures for TrxB

Using the SMART program, a TrxB functional domain (43 – 285 aa) was predicted (Fig. 5). Secondary structure prediction revealed following composition: random coils (31.97%), alpha-helices (31.66%), extended strands (25.08%), and beta turns (11.29%) (Fig. 6).

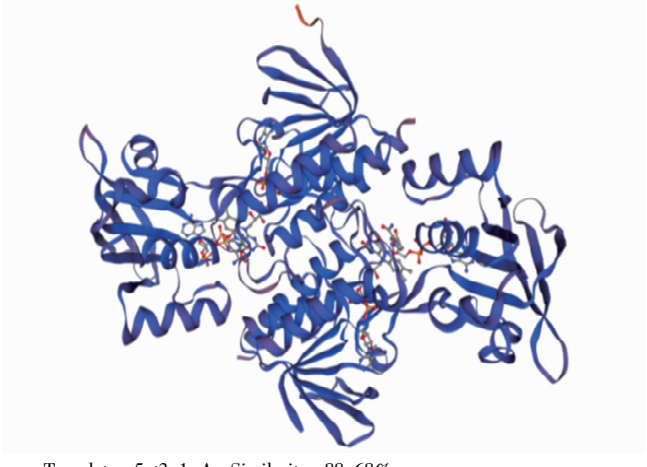
The amino acid sequence of TrxB was submited to SWISS-MODEL program, and homologous proteins were automatically searched as templates to obtain the single-subunit tertiary structure model of TrxB (Fig. 7).

Fig. 5 Functional domain of TrxB



Blue: Alpha helix; Purple: Random coil; Red: Extended strand; Green: Bate turn.

Fig. 6 Secondary structure prediction of TrxB



Template: 5vt3.1.A; Similarity: 88.68%.

Fig. 7 Tertiary structure prediction of TrxB

Protein-protein interaction network of TrxB

In the protein-protein interaction network, proteins adjacent to TrxB include trxA-2, tsaA-1, ahpC, trxC, ANP67598.1, ANP64013.1, ANP67564.1, ANP64424.1, ANP65389.1, and hmp (Fig. 8).

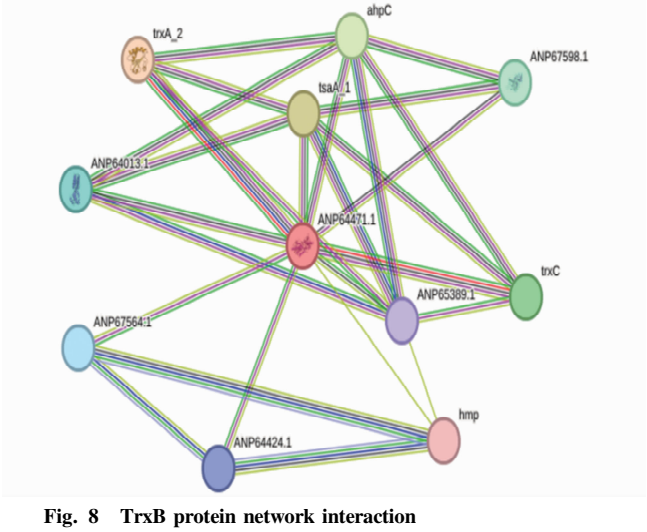


Fig. 8 TrxB protein network interaction

Conclusions and Discussion

In this study, the *trxB* gene from *V. alginolyticus* HY9901 were successfully cloned and analyzed, revealing multiple biochemical characteristics of its encoded protein. The TrxB protein was predicted to have a molecular weight of 34.32 kDa and an isoelectric point of 4.77, suggesting its specific stability and potential functions within cells. Bioinformatics analysis identified multiple phosphorylation sites and a signal peptide, indicating its potential roles in signal transduction and protein processing. Furthermore, the high probability of *trxB* localization in the endoplasmic reticulum points to its possible importance in protein folding and quality control. Homology analysis demonstrated high sequence conservation with *trxB* from other *Vibrio* species, reflecting its evolutionary conservation within the genus. These findings provide new insights into understanding the role of *trxB* in the pathogenic mechanisms of *V. alginolyticus*, laying a foundation for developing targeted prevention and control strategies against the bacterium.

In *V. alginolyticus*, Trx is a part of immune defense, which can promote the reduction of other proteins and act as an antioxidant[14]. Additionally, TrxR in *V. harveyi* can provide immunoprotective effects, and its expression in *E. coli* may confer immune protection in turbot (*Scophthalmus maximus*)[15]. This protein is also involved in selenium metabolic pathways. Microbial selenium metabolism primarily includes selenium transport, reduction, oxidation, assimilation, and methylation[16].

Overall, research on microbial selenium metabolism has achieved relatively clear understanding regarding selenium assimilation and reduction mechanisms. The assimilation pathways have been elucidated in both fungi and bacteria (including archaea)[17]. Specific selenate reductases have been identified in both Gram-negative and Gram-positive bacteria[18], though no specific selenite reductase or other key selenite reduction genes have been discovered to date. Selenium transport is associated with sulfate or phosphate transport systems and is also influenced by factors such as temperature, pH, carbon sources, and nitrogen sources. Several key genes and enzymes involved in selenium

methylation have also been identified. However, knowledge remains limited regarding selenium-oxidizing microorganisms, and systematic studies on microbial selenium oxidation mechanisms are still lacking. Therefore, our research group will focus future investigations on the role of the TrxB protein in selenium oxidation processes in *V. alginolyticus*.

In this study, the full-length *trxB* gene sequence was successfully amplified from *V. alginolyticus* HY9901. The gene is 960 bp in length and encodes 319 amino acids. Bioinformatics analysis revealed following characteristics of the protein:

(1) Physicochemical properties: The protein is unstable and hydrophobic, with a theoretical isoelectric point (pI) of 4.77 and a predicted molecular weight of 34.32 kDa.

(2) Structure and localization: No signal peptide or transmembrane domains were detected. Subcellular localization prediction indicated predominant mitochondrial localization.

(3) Functional sites: The amino acid sequence contains multiple phosphorylation sites and targeting signal sites, and there is a functional domain.

(4) Secondary and tertiary structures: The secondary structure consists mainly of random coils, alpha-helices, extended strands, and a small proportion of beta turns. The tertiary structure model showed 88.68% similarity to the template 5vt3.1.A.

(5) Homology analysis: The TrxB amino acid sequence of *V. alginolyticus* exhibited the highest homology (98.4%) with other *Vibrio* species, indicating their closest evolutionary relationship.

Through preliminary bioinformatics analysis, basic information and functional roles of the *trxB* gene in *V. alginolyticus* have been clarified, providing theoretical guidance for the subsequent development of new vaccines.

References

[1] YANG SL, WANG YG, DONG SG. Progress of research on vibriosis in marine cultured fish[J]. Marine Fisheries Research, 2005(4): 75 – 83. (in Chinese).

[2] LUAN YZ. Isolation and control of *Vibrio alginolyticus* in *Litopenaeus vannamei* culture system[D]. Qingdao: Qingdao University of Technology, 2023. (in Chinese).

[3] DA F. Pathogenic mechanism of two effector proteins of *Vibrio alginolyticus* T3SS and antibacterial effect of innate immune factors of *Trachinotus ovatus*[D]. Zhanjiang: Guangdong Ocean University, 2023. (in Chinese).

[4] CHEN Q, YAN QP, MA S. Progress on pathogenicity research of *Vibrio alginolyticus*[J]. Marine Sciences, 2006(8): 83 – 89. (in Chinese).

[5] LIU HY, WANG K, YAN WJ, *et al.* Research status of research on the role of thioredoxin 1 in cerebral ischemia[J]. The Chinese Journal of Clinical Pharmacology, 2024, 40(10): 1525 – 1529. (in Chinese).

[6] SHI SY, WANG QY. Research progress of the thioredoxin interacting protein/nucleotide binding and oligomerization domain-like receptor family pyrin domain-containing 3 signaling pathway and diabetes mellitus and its chronic complications[J]. Chinese Journal of Diabetes Mellitus, 2024, 16(11): 1294 – 1300. (in Chinese).

[7] DU ZY, XIA DF, GAO XY. Review on progress of thioredoxin reductase structure and tumor therapy[J]. Journal of Beijing University of Technology, 2024, 50(2): 246 – 260. (in Chinese).

[8] QIAO BW, XU H, DUAN WS. Protective effect of tetramethylpyrazine on acute lung injury mice through regulating txnip/TRX/NF-κB signaling pathway[J]. Journal of Emergency in Traditional Chinese Medicine, 2024, 33(7): 1174 – 1177. (in Chinese).

[9] ASHRAFULLAH, KHAN, ADNAN, KHAN, BUSHRA, SHAL, *et al.* Ameliorative effect of two structurally divergent hydrazide derivatives against DSS-induced colitis by targeting Nrf2 and NF-κB signaling in mice[J]. Naunyn-Schmiedeberg's Archives of Pharmacology, 2022, 395(10): 1167 – 1188. (in Chinese).

[10] WANG EW. Expression and functional analysis of scorpion neurotoxin AaIT[D]. Wuhan: Wuhan University, 2005. (in Chinese).

[11] HUANG ZJ, HE JG. Immunogenicity and immunoprotection of outer membrane proteins of *Vibrio alginolyticus*[J]. Journal of Fisheries of China, 2006, 30(4): 538 – 543.

[12] LIANG CF, PAN HY, LIU JY. Molecular cloning and bioinformatics analysis of *vscH* gene from HY9901 strain of *Vibrio alginolyticus*[J]. Journal of Zhanjiang Ocean University, 2018, 38(4): 85 – 91. (in Chinese).

[13] LIU X, CHEN C, CHEN CL, *et al.* The secondary structure, antigen epitope analysis and recombination prediction triple epitope peptide vaccine of porcine important infection virus protein[J]. Journal of Biology, 2017, 34(3): 18 – 23. (in Chinese).

[14] WU T. Large-scale identification of direct interaction proteins and substrates of oxidoreductase in living cells[D]. Hangzhou: Zhejiang University, 2023. (in Chinese).

[15] TAO R, LIU R, WANG Q, *et al.* Expression of thioredoxin reductase of *Vibrio harveyi* SF1 in bacterium *Enterobacter coli* and immunogenicity in turbot *Scophthalmus maximus*[J]. Journal of Dalian Fisheries University, 2012, 27(2): 137 – 142. (in Chinese).

[16] FENG JF, LI SL. Anti-cancer mechanism of selenium selenoprotein and selenium[J]. Studies of Trace Elements and Health, 2001(1): 70 – 72.

[17] HE YT, GUO QQ, YUE YC, *et al.* Expression and purification of thioredoxin glutathione reductase from *Fasciola gigantica* and preparation of polyclonal antibody[J/OL]. Chinese Journal of Animal Infectious Diseases, 1 – 11[2025 – 03 – 11]. (in Chinese).

[18] ZHOU XW. Study on the reduction of typical pollutants by extracellular polymer-mediated microbial extracellular electron transfer[D]. Nanjing: Nanjing University, 2020. (in Chinese).

Editor: Yingzhi GUANG Proofreader: Xinxiu ZHU