Cloning and Bioinformatics Analysis of *cobQ* Gene from *Vibrio alginolyticus* Strain HY9901

Chao HUANG, Zhihang CHEN, Weijie ZHANG, Xiaoxin WEN, Jiajie MA, Peng ZHOU, Yingying JIANG, Huanying PANG*

Fisheries College, Guangdong Ocean University, Zhanjiang 524025, China; 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

Abstract [**Objectives**] To analyze the function of *cobQ* gene from *Vibrio alginolyticus* strain HY9901, and to provide a reference for exploring the possible mechanism of *cobQ* gene from *V. alginolyticus*. [**Methods**] A pair of primers were designed based on the sequence of the *V. alginolyticus cobQ* gene and used to amplify the full-length gene by PCR. [**Results**] The PCR amplification results indicated that the *cobQ* gene has a full length of 780 bp, encoding 259 amino acid residues. The deduced amino acid sequence predicts a molecular weight of approximately 28.83 kD and an isoelectric point of 9.21. Sequence analysis revealed no N-terminal signal peptide cleavage site, suggesting the absence of both a signal peptide and transmembrane regions in this protein. The amino acid sequence contains 2 N-terminal myristoylation sites, 1 N-glycosylation site, 1 glycosaminoglycan attachment site, 4 microbody C-terminal targeting signal sites, 3 casein kinase II phosphorylation sites, and 4 protein kinase C phosphorylation sites. Subcellular localization prediction showed that the CobQ protein is primarily localized in the cytoplasm (65.2% probability). Homology analysis demonstrated that the amino acid sequence of the *cobQ* gene from *V. alginolyticus* shares up to 99% homology with other *Vibrio* species, clustering within the same subclade as *Vibrio parahaemolyticus*, indicating close phylogenetic relationships. Secondary structure prediction revealed proportions of α-helices, random coils, and extended strands as 44.40%, 36.68%, and 18.92%, respectively. The tertiary structure model exhibited 87.62% similarity to the template A0A165XBE1.1. [**Conclusions**] In this study, the *V. alginolyticus cobq* gene was successfully cloned and its sequence was analyzed by bioinformatics. It is expected to lay a foundation for the subsequent study of the regulatory mechanism of its protein on the virulence of *V. alginolyticus*.

Key words *Vibrio alginolyticus*, Gene cloning, *cobQ* gene, Bioinformatics analysis

0 Introduction

Vibrio alginolyticus, belonging to the family Vibrionaceae and genus *Vibrio*, is a Gram-negative short bacillus. This species exhibits an obligate halophilic nature and demonstrates broad tolerance to temperature and salinity. Its optimal growth temperature ranges from 30 to 36 ℃, with a preferred NaCl concentration of 2% to 4%. Growth of *V. alginolyticus* is inhibited under iron concentrations below 0.2 μM; however, its metabolism and growth can be promoted in environments with adequate iron levels[1-2]. Vibriosis caused by *V. alginolyticus* predominantly occurs in summer when water temperatures range between 28 to 35 ℃, with increased disease prevalence risks, particularly when aquatic animals experience weakened immunity or environmental deterioration[3]. *V. alginolyticus* possesses a polar flagellum and lateral flagella, enabling free motility in both liquid and solid media, and this motility plays a critical role in the dissemination of pathogen virulence[4-5]. At present, over ten pathogenic *Vibrio* species are known to cause severe harm to economically important aquatic animals, with *V. alginolyticus* being the primary pathogenic bacterium in mariculture industries in southern China's coastal regions[6]. This bacterium is widely distributed in marine and estuarine environments, exhibiting strong pathogenicity. Infected fish show high mortality rates, often presenting symptoms such as darkened body coloration, congestion of pectoral and ventral fins, and muscular ulceration. In addition, *V. alginolyticus* is a zoonotic pathogen[7], with coastal residents or individuals engaged in aquatic occupations being susceptible to skin and ear canal infections[8].

Protein acetylation, first discovered in eukaryotic histone lysine, is a reversible post-translational modification involved in almost all life activities[9]. In bacteria, common types of modifications include acetylation, methylation, succinylation, and ubiquitination. Acetylation plays an important role in the cellular physiology and function of bacteria, and regulates virulence. Succinylation is widely found in bacteria, closely related to molecular function and biological activity, and plays an important role in bacterial virulence and pathogenicity. Deacetylation modifications are essential for the regulation of protein function and cellular conduction. CobQ is a novel Zn^{2+}, NAD^+ and ATP independent deacetylase[10]. In *Aeromonas hydrophila*, it has been confirmed that CobQ has deacetylation modification function and desuccinylation modification function. Studies have shown that the CobQ protein of *Aeromonas hydrophila* affects many biological processes, such as protein transcription and translation, energy metabolism and microbial metabolism in different environments, by regulating

Received: January 9, 2025 Accepted: March 14, 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).

Chao HUANG, undergraduate, research fields: prevention and control of aquatic animal diseases.

* Corresponding author. Huanying PANG, doctoral degree, associate professor, research fields: prevention and control of aquatic animal diseases.

the expression of a variety of key enzymes. In addition, CobQ may have the ability to improve the adaptability of *A. hydrophila* under various environmental stresses[11]. Therefore, it is of great significance to further study the protein function of CobQ for clarifying the mechanism of energy utilization and pathogenesis of *V. alginolyticus*. In the related research of *A. hydrophila*, the protein substrate modified by CobQ may involve transcription and translation, energy metabolism, two-component system and other related proteins[10], but the protein substrate modified by CobQ in *V. alginolyticus* needs further study.

Although the deacetylation and desuccinylation modification functions of CobQ have been confirmed in *A. hydrophila*, there are few reports about related functions of CobQ in *V. alginolyticus*. In view of this, to further explore the functional mechanism of *V. alginolyticus cobQ* gene, we successfully cloned *V. alginolyticus cobQ* gene and analyzed its sequence by bioinformatics. It is expected to lay a foundation for the subsequent study of the regulatory mechanism of its protein on the virulence of *V. alginolyticus*.

1 Materials and methods

1.1 Materials

1.1.1 Strain and vector. *V. alginolyticus* HY9901[12] was a virulent strain preserved in our laboratory; *E. coli* DH5α competence was preserved in our laboratory; cloning vector pMD18-T was purchased from Takara Company.

1.2 Methods

1.2.1 Extraction of total DNA from *V. alginolyticus* HY9901. With reference to the method of Zeng Fuyuan[13], we extracted genomic DNA according to the kit instructions and stored at −20 ℃.

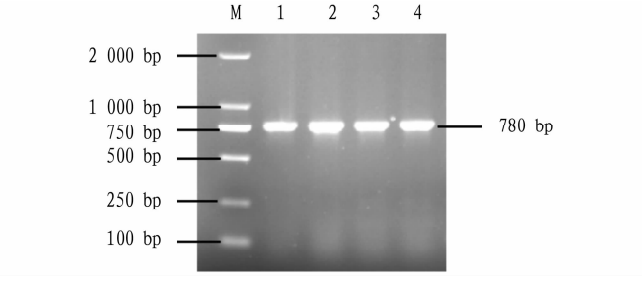
1.2.2 Cloning of *cobQ* gene. We designed a pair of primers according to the genetic sequence *cobQ* of *V. alginolyticus*. The upstream primer P1 was ATGATTGTTTGGAGTGTAGC, and the downstream primer P2 was TTACTGC TCATCGAACGCCAACCTC. The total DNA of *V. alginolyticus* HY9901 was used as template, and the PCR reaction conditions were as follows: pre-denaturation at 98 ℃ for 3 min, denaturation at 98 ℃ for 30 sec, annealing at 54 ℃ for 30 sec, extension at 72 ℃ for 40 sec, 33 cycles, and extension at 72 ℃ for 5 min. The PCR products were detected by 1% agarose gel electrophoresis and finally recovered.

1.2.3 Sequencing of PCR products. According to the instructions, the PCR product was ligated to the pMD18-T vector and then transformed into DH5α competent cells to facilitate its entry and stable presence in these cells. It was spread on the LB plate containing $Kana^+$ resistance to select the colonies successfully transformed and carrying the target plasmid, and sent to Guangzhou Sangon Biotech Company for determination.

1.2.4 Bioinformatics analysis of *V. alginolyticus cobQ* gene. We carried out this experiment using the method of Pang Huanying[14]. Sequence homology comparison and similarity analysis are carried out utilizing an NCBI website; physical and chemical properties are analyzed by ExPASy Proteomics Server; nucleic acid homology comparison analysis is carried out using DNAMAN Version 6.0; an open reading frame is determined using ORF Finder; Signal peptide sequence was predicted by online analysis software SignalP 5.0 Server; functional site distribution in amino acid sequence was predicted by SoftBerry-P site; transmembrane domain was predicted by TMHMM Server 2.0; lactate modification site was predicted by Kla website; SMART website was used to analyze the functional domain of protein structure; Gneg-PLoc software was used to predict the subcellular localization; Clastal 2.0 and MEGA 5.0 software were used to construct the phylogenetic tree; SWISS-MODEL program of ExPASy server was used to model; KEGG website was used to find the metabolic pathway; the STRING database was searched for protein network interactions; the musite website was used for PTM modification site prediction analysis.

2 Results and analysis

2.1 Amplification of *cobQ* gene A specific band of about 780 BP was successfully amplified by PCR (Fig. 1), and the gene encodes 259 amino acids. NBCI access number was PQ351403.



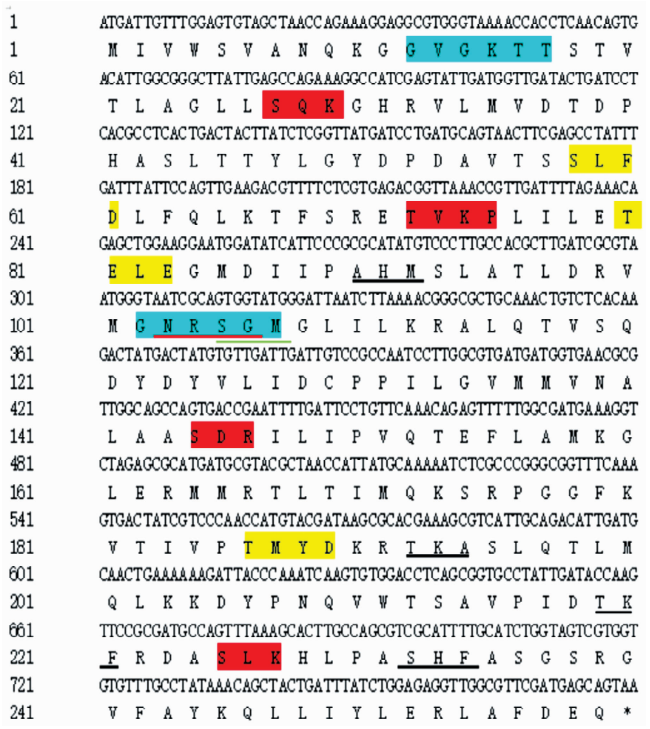
NOTE M: DL2000 DNA marker; 1 − 4: amplification results of *cobQ* gene.

Fig. 1 Cloning of *cobQ* gene

2.2 Physical and chemical properties of *cobQ* gene With the aid of ExPASy software, we analyzed the CobQ protein of *V. alginolyticus*, and the results showed that the total number of atoms was 4 108, and the molecular structural formula is $C_{1\,291}H_{2\,066}N_{342}O_{372}S_{15}$. The theoretical molecular weight is 28.833 75 kDa and the theoretical *pI* value is 9.21. The instability coefficient was 29.60 (stable), the fat coefficient was 97.94, the total average hydrophilicity was 0.023, and the overall protein was hydrophilic. This protein did not contain pyrrolysine (Pyl) and selenocysteine (Sec) and exhibited a molar extinction coefficient of 22 920 (mol/cm) at 280 nm. The total number of acidic amino acids (Asp + Glu) was 24, the total number of basic amino acids (Arg + Lys) was 29, and the N-terminal was methionine (Met). The half-life of expression in yeast and *E. coli* was more than 20 h and 10 h, respectively, and the half-life of expression in mammalian reticulocytes *in vitro* was 30 h.

2.3 Sequence analysis of *cobQ* (Fig. 2) Through the Signal P 4.0 Server program, it predicted the N-terminal signal peptide structure of CobQ amino acid sequence, and found that there was no obvious signal peptide cleavage site and no signal peptide. The protein was predicted to have no transmembrane domain by TMHMM Server 2.0 program. Sequence analysis using SoftBerry-Psite predicted that the amino acid sequence contained two N-terminal myristoylation sites (12 − 17 aa, 102 − 107 aa), one N-glycosylation site (103 − 106 aa), one glycosaminoglycan attachment site (105 − 108 aa), and one N-glycosylation site (105 − 108 aa);

four microbody C-terminal target signal sites (27 – 29 aa, 72 – 74 aa, 144 – 146 aa, 225 – 227 aa), three casein kinase II phosphorylation sites (58 – 61 aa, 80 – 83 aa, 186 – 189 aa); four protein kinase C phosphorylation sites (90 – 92 aa, 192 – 194 aa, 219 – 221 aa, 232 – 234 aa). The results of protein subcellular localization prediction showed that CobQ was most likely to be located in the cytoplasm (65.2%), followed by mitochondria and nucleus (17.4% and 13.0%, respectively), while the possibility of protein subcellular localization in the cytoskeleton was 4.3%.

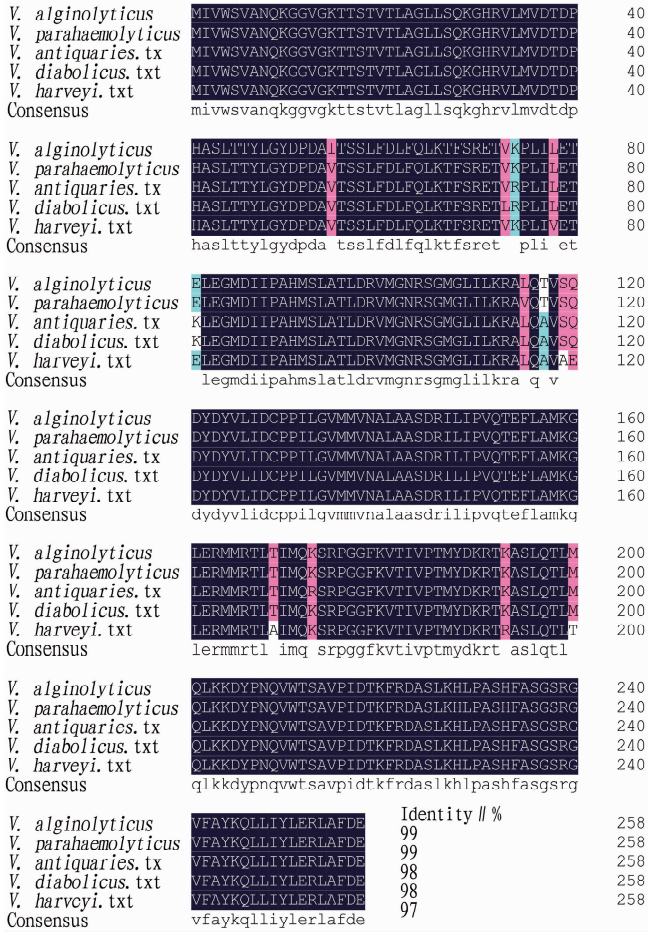


NOTE Terminator is indicated by symbol *; N-terminal myristoylation site is indicated by light blue background; protein kinase C phosphorylation site is indicated by black underline; casein kinase II phosphorylation site is indicated by yellow background; N-glycosylation site is indicated by red underline; microbody C-terminal localization signal site is indicated by red background; glycosaminoglycan attachment site is indicated by green underline.

Fig. 2 Nucleotides of the *cobQ* gene and the amino acid sequences they encode

2.4 Homology and evolution analysis of *cobQ* BLAST analysis showed that the *cobQ* of *V. alginolyticus* had high homology with the *cobQ* of other *Vibrio*, and the highest homology with the *cobQ* of *V. parahaemolyticus* was 99%. Multiple sequence similarity comparison showed that the *cobQ* in *Vibrio* was highly conserved (Fig. 3).

Using MEGA 5.0 software, we constructed the phylogenetic tree by Neighbor-joining (NJ) method. The results showed that the CobQ proteins of *V. alginolyticus* and *V. parahaemolyticus* were clustered into the same subgroup. It shows that there is a close relationship between them (Fig. 4), which is consistent with the traditional classification of morphological and biochemical properties.



NOTE *V. alginolyticus* (WP_005377193.1); *V. parahaemolyticus* (WP_072847685.1); *V. antiquaries* (WP_074191268.1); *V. diabolicus* (WP_048626465.1); *V. harveyi* (WP_025768113.1).

Fig. 3 Comparison of homology of amino acid sequences of *cobQ* genes

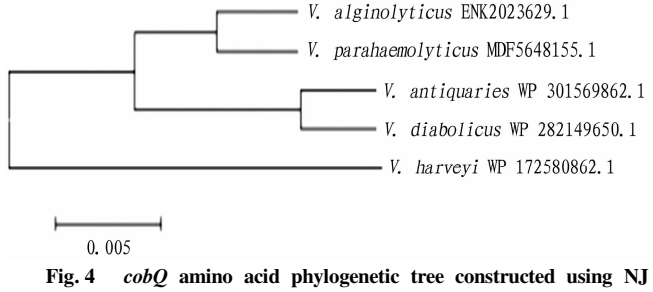
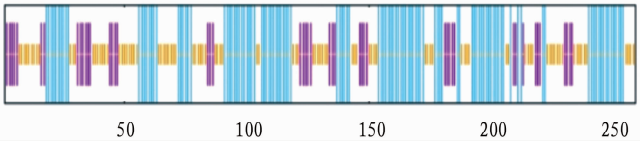


Fig. 4 *cobQ* amino acid phylogenetic tree constructed using NJ method

2.5 Functional domain, secondary and tertiary structure prediction of *cobQ* Using SMART program to predict, one Pfam CBP-BcsQ functional domain (1 – 168 aa) was found (Fig. 5). Alpha helix accounted for 44.40% in the secondary structure prediction, random coil accounted for 36.68%; extended strand accounted for 18.92% (Fig. 6).

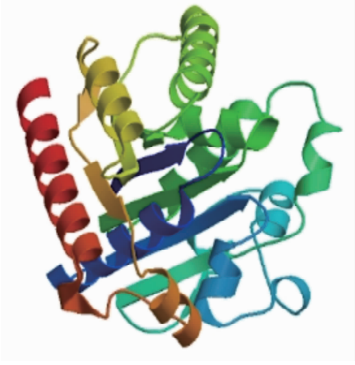
Then, we imported the amino acid sequence of CobQ into the SWISS-MODEL program, and homologous proteins were automatically searched as templates to obtain a single subunit tertiary structure model of CobQ (Fig. 7).

Fig. 5 Functional domain of CobQ protein



NOTE Blue: alpha helix; yellow: random coil; purple: extended strand.

Fig. 6 Secondary structure of CobQ protein



NOTE Template: A0A165XBE 1.1; similarity: 87.62%.

Fig. 7 3D structural model of CobQ protein subunit of *Vibrio alginolyticus*

2.6 CobQ protein-protein interaction (PPI) network In the PPI network, it can be found that the proteins adjacent to CobQ were btuB, virB, ANP66210.1, ANP65348.1, flhG, ANP66486.1, ANP65344.1, ANP65347.1, cheB1 and cheW.

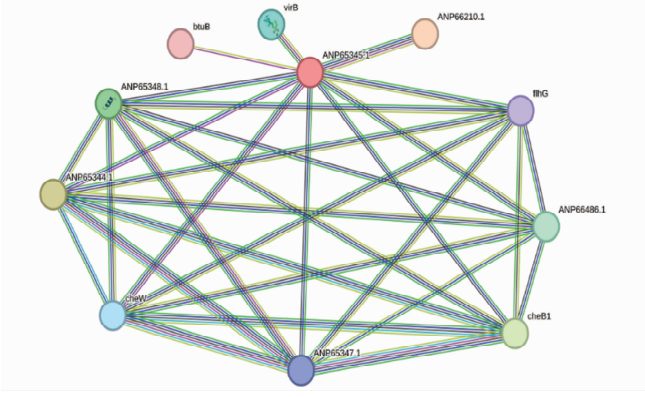


Fig. 8 CobQ PPI network

2.7 Prediction of PTM modification sites of copQ PTM modification sites were predicted and analyzed by musite website (MusiteDeep) and FSL-Kla (http://kla.zbiolab.cn/) program, and *cobQ* amino acid sequence was submitted for automatic analysis. Phosphorylation, Glycosylation, Acetylation, and pyrrolidone carboxylic acid (pyrrolidone carboxylic acid) modification sites were found.

3 Discussion

In this study, the amino acid sequence of *cobQ* gene from *V. alginolyticus* was used to predict the possible mechanism of CobQ protein by comprehensive comparison and analysis of its secondary structure, tertiary structure, hydrophilicity, transmembrane region and protein network interaction.

Bioinformatics analysis revealed that CobQ protein was composed of leucine, threonine, valine, alanine, serine and other amino acids, indicating that the structure of CobQ protein was complex and stable. Protein stability refers to its ability to maintain its native conformation, while protein instability can easily lead to misfolding and loss of function under external environmental pressure. High stability helps the protein to perform its function accurately[15]. According to the prediction of protein subcellular localization, CobQ protein may exist in the cytoplasm, mitochondria, nucleus, cytoskeleton and other subcellular structures. The functions of subcellular structures are performed by their internal proteins, and proteins must be transported to the corresponding subcellular locations to perform their functions correctly[16]. This suggests that CobQ protein may be involved in metabolism, energy utilization, cell cycle and other life activities in cells. CobQ protein did not show obvious signal peptide and transmembrane region in the N-terminal signal peptide structure prediction analysis, suggesting that the protein may rely on molecular chaperones to enter the membrane system to exert its function. Proteins are prone to misfolding in the process of folding due to marginality. Molecular chaperones can participate in the folding of new peptides, correct misfolding, refold proteins, and recognize and degrade proteins with abnormal functions, thus playing a key role in maintaining protein stability[17]. In the related research of *Pichia pastoris*, molecular chaperones can also improve the expression of proteinase K[18]. It is important to study the molecular chaperone of CobQ protein for revealing the function and stability of CobQ protein. The deduced amino acid sequence of CobQ shared 95% identity with that of *V. parahaemolyticus* and *V. harveyi*, and 99% identity with that of *V. parahaemolyticus*, indicating that the amino acid sequence of CobQ was highly conserved in *Vibrio* and widely distributed in *Vibrio*, which may be involved in a variety of important life activities, such as metabolism, transcription and translation.

According to the prediction of PTM modification sites, CobQ protein has a variety of modification sites, including acetylation, glycosylation, phosphorylation and pyrrolidone carboxylation. There may be interactions between different PTMs, that is, post-translational modifications at one site may affect the function of another site, a phenomenon known as ectopic crosstalk[19]. In ectopic crosstalk, the effect of one modification site on another modification site may be positive, for example, Swaney *et al.* found crosstalk between phosphorylation and ubiquitination in protein degradation, and phosphorylation can promote subsequent ubiquitination[20]. However, the specific positive or negative effects between the PTM modification sites of *cobQ* gene need to be further studied. CobQ protein has multiple functional sites, including an N-terminal myristoylation site, an N-glycosylation site, a glycosaminoglycan attachment site, a microbody C-terminal target sig-

naling site, a casein kinase II phosphorylation site, and a protein kinase C phosphorylation site, indicating that CobQ may be involved in a variety of life activities. Glycosylation modification can not only regulate the structural stability of proteins themselves, but also affect protein functions such as cell adhesion and intercellular signaling[21]. Phosphorylation modification is a common reversible post-translational modification, which is involved in regulating protein folding, changing protein structure, and then affecting protein function, such as affecting the activity and substrate specificity of related enzymes, regulating cell cycle, cell differentiation, development, apoptosis and other life activities[22].

At present, the research on *cobQ* gene and CobQ protein is mainly focused on *A. hydrophila*. Wang Yuqian *et al.* [15] believed that CobQ protein is a novel deacetylase independent of ATP, Zn^{2+} and NAD^{+}. In *A. hydrophila*, CobQ is involved in the regulation of many physiological functions, such as transcription and translation, energy metabolism, virulence factors, and so on, while the motility and iron utilization ability of *A. hydrophila* are significantly improved after the absence of CobQ, indicating that CobQ has a negative regulatory role in *A. hydrophila*, affecting the ability of bacteria to use energy and adapt to the environment. Therefore, CobQ is involved in the regulation of a variety of life activities, but the specific mechanism of action in *V. alginolyticus* is still not clear, and future research is expected to fill this gap.

4 Conclusions

The *cobQ* gene has a full length of 780 bp, encoding 259 amino acid residues. Its molecular weight is about 28.83 kD and isoelectric point is 9.21. The protein has no obvious signal peptide cleavage site, no signal peptide and no transmembrane region. The amino acid sequence contains two N-terminal myristoylation sites, one N-glycosylation site, one glycosaminoglycan attachment site, four microbody C-terminal target signal sites, three casein kinase II phosphorylation sites, and four protein kinase C phosphorylation sites. CobQ protein is most likely to be located in the cytoplasm. The amino acid sequence of *V. alginolyticus* and *V. parahaemolyticus* CobQ had the highest homology of 99%, indicating that they were very close in evolutionary relationship. It has one Pfam CBP-BcsQ functional domain. In the secondary structure, α-helix and random coil were the main parts with a few extended strands. The similarity of the tertiary structure model to the template A0A165XBE1.1 was 87.62%. The proteins interacting with CobQ protein include btuB, virB, ANP66210.1, ANP65348.1, flhG, ANP66486.1, ANP65344.1, ANP65347.1, cheB1 and cheW. In *A. hydrophila*, *cobQ* can regulate a variety of biological processes, such as energy utilization, movement, virulence factors, *etc.*, while in *V. alginolyticus*, the mechanism of *cobQ* is not yet clear, and the analysis of biological information will provide a theoretical basis for subsequent research.

References

[1] JIA AR. Cloning and expression analysis of four immune-related genes and hemolysin of *Vibrio alginolyticus* in Scophthalmus maximus [D]. Qingdao: Ocean University of China, 2011. (in Chinese).

[2] NORFOLK WA, SHUE C, HENDERSON WM, *et al.* *Vibrio alginolyticus* growth kinetics and the metabolic effects of iron [J]. Microbiology Spectrum, 2023, 11(6): e0268023.

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

[4] QIAN RH. Cloning, expression and immunogenicity of major virulence related genes of *Vibrio Alginolyticus* [D]. Hangzhou: Zhejiang University, 2008. (in Chinese).

[5] ZHU S, NISHIKINO T, HU B, *et al.* Molecular architecture of the sheathed polar flagellum in *Vibrio alginolyticus* [J]. Proceedings of National Academy of Sciences, 2017, 114(41): 10966-10971

[6] RUI HP. From fish to human: studies on pathogenesis of major pathogenic *Vibrio* [D]. Shanghai: East China University of Science and Technology, 2011. (in Chinese).

[7] RIPABELLI G, SAMMARCO ML, GRASSO GM, *et al.* Occurrence of Vibrio and other pathogenic bacteria in *Mytilus galloprovincialis* (mussels) harvested from Adriatic Sea, Italy [J]. International Journal of Food Microbiology, 1999, 49(1): 43-48.

[8] JACOBS SLIFKA KM, NEWTON AE, MAHON BE. *Vibrio alginolyticus* infections in the USA, 1988-2012 [J]. Epidemiology and Infection, 2017, 145(7): 1491-1499. (in Chinese).

[9] LIU Q, SHAO L. Protein acetylation in bacteria [J]. Microbiology China, 2021, 48(2): 637-647. (in Chinese).

[10] WANG YQ. Identification and functional characterization of a novel bacterial Deacetylase AhCobQ [D]. Fuzhou: Fujian Agriculture and Forestry University, 2023. (in Chinese).

[11] WANG GB. Proteomics and desuccinylation of *Aeromonas hydrophila* CobQ [D]. Fuzhou: Fujian Agriculture and Forestry University, 2023. (in Chinese).

[12] CAI SH, WU ZH, JIAN JC, *et al.* Cloning and expression of gene encoding the thermostable direct hemolysin from *Vibrio alginolyticus* strain HY9901, the causative agent of vibriosis of crimson snapper (Lutjanus erythopterus) [J]. Journal of Applied Microbiology, 2007, 103(2): 289-296.

[13] ZENG FU. Effect of lysine succinylation of PEPCK on drug resistance of *Vibrio alginolyticus* [D]. Zhanjiang: Guangdong Ocean University, 2022. (in Chinese).

[14] PANG HY, ZHOU ZJ, DING J, *et al.* Molecular cloning and bioinformatics analysis of T3SS chaperone escort protein VscO from *Vibrio alginolyticus* [J]. Biotechnology Bulletin, 2014(6): 155-161. (in Chinese).

[15] RUAN QY, HUANG SHEN, MENG ZJ, *et al.* Computational design and directed evolution strategies for optimizing protein stability [J]. Synthetic Biology Journal, 2023, 4(1): 5-29. (in Chinese).

[16] QIAO SP, YAN BQ. Review of protein subcellular localization prediction [J]. Application Research of Computers, 2014, 31(2): 321-327. (in Chinese).

[17] TANG YQ, YE ST, LIU J, *et al.* Molecular chaperones promote protein stability and evolution [J]. Synthetic Biology Journal, 2022, 3(3): 445-464. (in Chinese).

[18] CAI YA, ZHANG YQ, YANG ZX, *et al.* Enhanced expression of protease K in *Pichia pastoris* through molecular chaperones and analysis of its effect on wool scale layer [J]. Biotechnology Bulletin, 2024, 40(7): 307-313. (in Chinese).

[19] SHA YT. Ensemble learning-based prediction and investigation of "in situ crosstalk" sites modified by serine phosphorylation and ADP-ribosylation [D]. Qingdao: Qingdao University, 2023. (in Chinese).

[20] SWANEY DL, BELTRAO P, STARITA L, *et al.* Global analysis of phosphorylation and ubiquitylation cross-talk in protein degradation [J]. Nature Methods, 2013, 10(7): 676-682.

[21] LIU ZJ, QIAO L, YE ZY, *et al.* Identification of the glycosylation sites of Opsin3 and its glycosylation modification function [J]. Chinese Journal of Biotechnology, 2022, 38(3): 1173-1182. (in Chinese).

[22] BAI X. Phosphoproteomics of *Clostridium acetobutylicum* [D]. Beijing: Beijing Institute of Genomics, Chinese Academy of Sciences, 2016. (in Chinese).