Molecular Cloning and Bioinformatics Analysis of *sucC* Gene of *Vibrio alginolyticus* Strain HY9901

Yingzhu WEI[1,2], Zhiqing WEI[1,2], Xuelian LIN[1,2], Huanying PANG[1,2*], Na WANG[3]

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. Chinese Academy of Inspection and Quarantine, Beijing 100176, China

Abstract [**Objectives**] To clone the *sucC* gene of *Vibrio alginolyticus* strain HY9901 and conduct the bioinformatics analysis. [**Methods**] Based on the *sucC* gene of *V. alginolyticus* strain HY9901, specific primers were designed to amplify the full length sequence by PCR and make further analysis. [**Results**] The theoretical molecular weight of SucC protein was about 41 528.45 Da, and the full length was 1 167 bp, encoding 388 amino acids. It has no signal peptide and transmembrane region, and has a variety of functional sites. It is predicted that it is mainly located in the cytoplasm, and the ubiquitin and lactate modification sites overlap, and it has high gene homology with Vibrio parahaemolyticus. The α-helix, random coil and extended strand are the main secondary structures. The similarity between the constructed three-level structure model and the template is high. [**Conclusions**] This study reveals the structural characteristics and functional potential of SucC protein, and provides a theoretical basis for the study of drug resistance mechanism and prevention strategies.

Key words *Vibrio alginolyticus*, Gene amplification, *sucC* gene, Succinyl-Coa synthetase, Protein post-translational modification, Bioinformatics analysis

1 Introduction

Vibrio alginolyticus, belonging to the genus *Vibrio* in the family Vibrionaceae, is a short-rod-shaped bacterium. *V. alginolyticus* is an important zoonotic pathogen. As a main opportunistic pathogen of vibriosis, it endangers many mariculture animals. Antibiotics play a central role in prevention and treatment, but drug resistance appears, and *sucC* gene plays a significant role in drug resistance. *V. alginolyticus* is a Gram-negative bacterium with mesophilic characteristics. Its pathogenic season is mostly warm summer, but it is not prone to get sick in winter[1]. In this experiment, it was found that the highest activity was obtained at pH 7.2, temperature 28 ℃, and culture time 18 h. *V. alginolyticus* is widely distributed in bays, estuaries and marine animals, it can cause diseases of fishes, shrimps and other marine animals, and can also infect humans, causing human diarrhea, post-traumatic infection, otitis media and other diseases[2-3]. For example, *V. alginolyticus* can cause vibriosis of marine animals such as *Lates calcarifer*, *Pagrus major*[4] and *Epinephelus akaara*, *Epinephelus fario*, *Lutjanus sanguineus*[5-6], and accordingly causing economic losses. Antibiotics are the main means to prevent and control vibriosis in aquaculture production, and play a huge role in the prevention and control of vibriosis[7]. However, with the widespread use of antibiotics, bacteria gradually develop resistance to drugs, which not only weakens the effect of drug prevention and control, but also brings some hidden dangers to the environment and food safety. In view of the increasingly serious drug resistance of *V. alginolyticus*, the mechanism of drug resistance has become a hot issue in academic circle. At present, the known drug resistance mechanisms of *V. alginolyticus* can be summarized as cell membrane permeability change, target protein modification, biofilm formation, active drug transport system and bypass metabolic compensation pathway[8-9], but the specific mode of action of these drug resistance mechanisms remains to be further studied.

During the development of drug resistance in *V. alginolyticus*, except for some resistance-related proteins (such as OmpC, OmpF and TolC, *etc.*), the expression of some key metabolic pathway-related proteins and enzyme activities will decrease significantly, such as isocitrate dehydrogenase (ICD), succinyl-Coa synthetase (SucC, SucD) and pyruvate dehydrogenase complex E1 (Odp1). These proteins are involved in the TCA cycle, pyruvate metabolism, and glycolysis, suggesting that in addition to common resistance mechanisms, bacteria can also affect key metabolic pathways to develop antibiotic resistance through down-regulating central carbon and energy generation[10-11]. Among them, Succinyl coenzyme A synthetase (SCS) is the key enzyme that catalyzes the only substrate-level phosphorylation step in the TCA cycle[12]. Fraser *et al.*[13] found that when succinyl-Coa synthetase of *Pseudomonas fluorescens* was overexpressed, it could form a fine metabolic balance, which made *P. fluorescens* resistant

Received: May 5, 2024 Accepted: July 9, 2024
Supported by National Natural Science Foundation of China (32073015); Graduate Education Innovation Program of Guangdong Province (YJYH[2022]1); Undergraduate Innovation and Entrepreneurship Training Program of Guangdong Ocean University (CXXL2024007); Undergraduate Innovation Team of Guangdong Ocean University (CCTD201802).
Yingzhu WEI, master candidate, research fields: aquatic veterinary medicine. * Corresponding author. Huanying PANG, PhD., associate professor, research fields: prevention and control of aquatic animal diseases.

to environmental toxins. Zeng Fuyuan[14] previously studied the protein succinylation modification of wild strains and drug-resistant strains of *V. alginolyticus*, and found that sucC was involved in the drug-resistant process of *V. alginolyticus*, suggesting that PTMs of the protein were involved in the drug-resistant regulation of *V. alginolyticus*, which laid a foundation for the study of the drug-resistant function of the protein. Although the role of *sucC* in bacteria has been studied, there are relatively few studies on the specific regulatory mechanism of *sucC* involved in *V. alginolyticus*, and the specific role of SucC protein in mediating drug resistance in *V. alginolyticus* is not clear. In this study, the *sucC* gene of *V. alginolyticus* strain HY9901 was cloned and bioinformatics analysis was carried out, which has a certain reference significance for further study of its function in bacterial resistance.

2 Materials and methods

2.1 Experimental materials

2.1.1 Strain. The virulent strain HY9901 of *V. alginolyticus* was obtained by our laboratory from the diseased *Lutjanus erythropterus* in the sea area of Zhanjiang, Guangdong Province[15].

2.1.2 Main reagents. ExTaq DNA polymerase and pmMD18-T vector were obtained from Takara Company; DNA extraction kit and gel recovery kit were purchased from TIANGEN Biotech (Beijing) Co., Ltd.; PCR primer synthesis and sequencing were obtained from Sangon Biotech Company. The concentration of antibiotics was ampicillin (Amp) 100 μg/mL.

2.2 Experimental methods

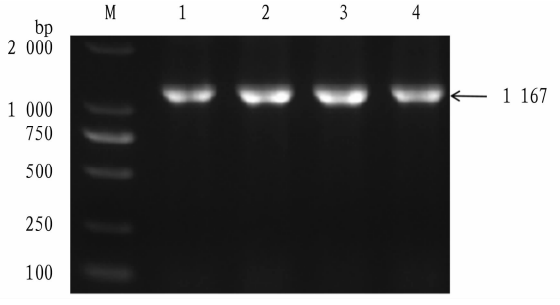
2.2.1 Extraction of total DNA of *V. alginolyticus* strain HY9901. A single colony of *V. alginolyticus* strain HY9901 was inoculated into TSB (5% NaCl) medium and cultured at 28 ℃ with shaking for more than 12 h, then centrifuged and thalli were collected. Genomic DNA of *V. alginolyticus* strain HY9901 was extracted in accordance with the instructions of DNA extraction kit, and stored in a refrigerator at −20 ℃ for later use.

2.2.2 Cloning of *sucC* gene. A pair of primers is designed according to the sequence of the *sucC* gene of the *V. alginolyticus*, the upstream primer S1 was ATGAATTTGCATGAATACCA, and the downstream primer S2 was TTATTTAGCCTCCGCAG. The total DNA of *V. alginolyticus* strain HY9901 was extracted as template, and the PCR reaction conditions were as follows: 94 ℃ predenaturation for 5 min; 94 ℃ for 30 sec; 50 ℃ for 30 sec; 72 ℃ for 40 sec; a total of 35 cycles, followed by 72 ℃ extension for 10 min. The PCR products were detected by agarose gel electrophoresis, and then were recovered according to the instructions of DNA recovery kit. The recovered fragments were cloned into pMD18-T vector, named pMD-sucC.

2.2.3 Bioinformatics analysis of *sucC* gene of *Vibrio alginolyticus* strain HY9901. Sequence homology analysis was performed using the NCBI website[16]; amino acid alignment analysis was performed using DNAMAN Version 6.0 (Lynnon Biosoft); ORF Finder and ExPASy Proteomics Server (http://ca.expasy.org) were used to deduce the amino acid sequence, and the physical and chemical properties such as molecular formula, total number of atoms, calculated molecular weight (Mw) and theoretical isoelectric point (pI) were analyzed[17]; signal peptide sequence was predicted by online analysis software SignalP 4.0 Server[18]; transmembrane domain was predicted by TMHMM Server 2.0[19]; SoftBerry-Psite was used to predict the distribution of functional sites of amino acids by sequence and structure analysis; the protein post-translational modification sites were predicted using MusitDeep[20]; lactate modification sites were predicted using FSL-Kla[21]; protein structural functional domains were analyzed using SMART program; the PRABI-GERLand website was used for secondary structure prediction, and Clastal 2.0 and MEGA 5.0 software were used to make phylogenetic trees by neighbor-joining method[22]. 3D modeling was performed and analyzed by the SWISS-MODEL[23] program of ExPASy; protein network interactions were studied using the STRING database (https://cn.string-db.org/)[24].

3 Results and analysis

3.1 Cloning of *sucC* gene A specific band of about 1 167 bp was successfully amplified by PCR (Fig. 1), and the gene encodes 388 amino acids. The accession number in GenBank is PP477066.1.

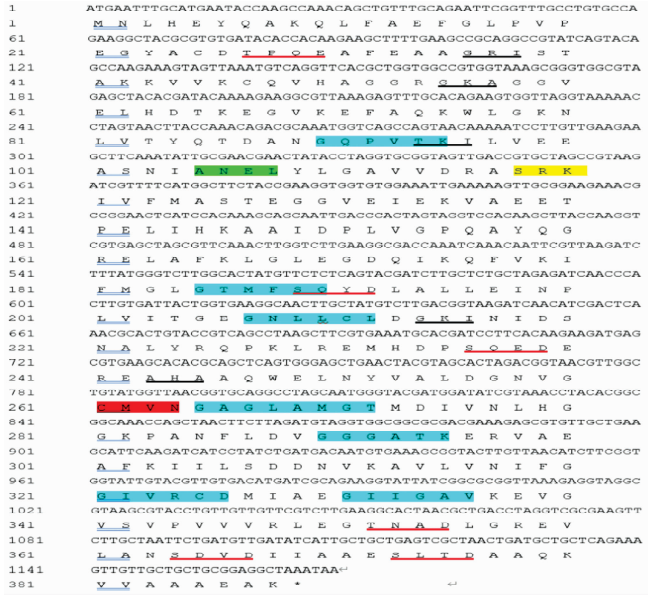


NOTE M: DL2000 DNA molecular weight standard; lanes 1-4: SucC PCR products.

Fig. 1 Amplification of *sucC* gene

3.2 Physical and chemical properties of SucC protein ExPASy software was used to analyze the physical and chemical properties of SucC protein of *V. alginolyticus* strain HY9901. The results showed that the molecular structure of SucC protein is $C_{1\,837}H_{2\,947}N_{499}O_{566}S_{14}$, and the total number of atoms is 5 863. The theoretical molecular weight is 41 528.45 Da and the theoretical isoelectric point is 4.90. The instability coefficient was 26.52, the fat coefficient is 98.56, and the average hydrophilicity is −0.024. The protein contains no selenocysteine (Sec) and pyrrolysine (Pyl), and exhibits a molar extinction coefficient of 23 170 (L/mol · cm) at 280 nm. The protein contains 55 acidic amino acids (Asp + Glu) and 37 basic amino acids (Arg + Lys), and its N-terminal is methionine (Met). The half-life of expression in yeast was more than 20 h, in *Escherichia coli*, the half-life of expression was more than 10 h, and in mammalian reticulocytes was 30 h. These results indicate that SucC protein is a relatively stable hydrophilic protein, which can also have good physical and chemical properties under adverse conditions.

3.3 Sequence analysis of SucC protein The N-terminal signal peptide, cleavage site and transmembrane domain of SucC amino acid sequence were predicted by SignalP 5.0 Server program and TMHMM Server 2.0 program, and it was found that there was no signal peptide and no transmembrane domain. SoftBerry-Psite predicted that the amino acid sequence contained 8 N-terminal myristoylation sites, 6 casein kinase II phosphorylation sites, 5 microbody C-terminal target signal sited, one protein kinase C phosphorylation site, one pentenyl binding site, and one endoplasmic reticulum signal peptide site (Fig. 2). The results of protein subcellular localization prediction showed that SucC was most likely to be located in the cytoplasm (60.9%), followed by the nucleus and mitochondria (17.4% and 13.0%, respectively), while only 4.3% of SucC was located in vacuoles and vesicles. These results indicate that SucC protein is a non-secretory and non-transmembrane protein, and it may play a major biological function in the cytoplasm. This will lay a theoretical foundation for further study of SucC protein function.



NOTE Terminators are indicated by *; blue background represents N-terminal myristoylation sites; red underlined represents casein kinase II phosphorylation sites; yellow background represents protein kinase C phosphorylation sites; red background represents pentenyl binding sites; black underlined represents microbody C-terminal localization signal sites; green background represent that signal peptide site of the endoplasmic reticulum.

Fig. 2 Nucleotide sequence of *sucC* gene and amino acid sequence encode by it

MusiteDeep is a deep learning framework for predicting general and kinase-specific phosphorylation sites, and it has outstanding advantages in PTM site prediction[25], and FSL-Kla is a multi-feature hybrid system for predicting lactate modification sites. Through prediction by MusiteDeep and FSL-Kla, the amino acid sequences have modification sites of ubiquitination, acetylation, methylation and lactylation. Among them, four ubiquitination modification sites overlapped with lactate modification sites, indicating that there may be crosstalk between them, which together affect some biological functions of SucC protein, but the mechanism of their interaction needs further study.

3.4 Homology and evolution analysis of SucC Through BLAST analysis, it was found that there was high homology between SucC of *V. alginolyticus* and other *Vibrio*, strains, among which the homology with *Vibrio parahaemolyticus* reached 99%, and the multiple sequence similarity comparison showed that SucC in *Vibrio* was highly conserved (Fig. 3).

NOTE *Vibrio alginolyticus* (WP_225473936.1); *Vibrio parahaemolyticus* (WP_005455468.1); *Vibrio diabolicus* (WP_258676161.1); *Vibrio maerlii* (WP_117233380.1); *Vibrio harveyi* (WP_085320100.1).

Fig. 3 Homology comparison of deduced amino acid sequences of sucC gene

With the aid of MEGA 5.0, the deduced amino acid sequence of SucC was compared with other *Vibrio* strains based on the Neighbor – Joining method. The results showed that the SucC protein of *V. alginolyticus* HY9901 and *V. parahaemolyticus* were clustered into the same subfamily (Fig. 4), indicating that they had a close relationship and a certain degree of similarity in biological function.

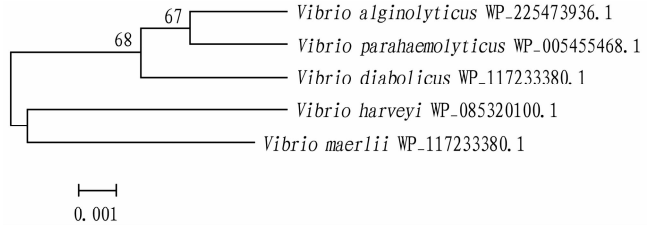


Fig. 4 SucC amino acid phylogenetic tree constructed based on Neighbor – Joining method

3.5 Functional domain, secondary and tertiary structure prediction of SucC The prediction results of the SMART program showed that the functional domains of the three SucCs were 1-218 AA, 2-203 AA, and 262-382 AA (Fig. 5 – 6). In the secondary structure prediction, SucC protein was composed of 46.65% of an α-helix, 22.42% of random coil, and 20.10% of extended strand (Fig. 7), indicating that SucC protein is mainly composed of an α-helix structure and it may play an important role in the hydrolysis of ATP and the synthesis of macromolecules.

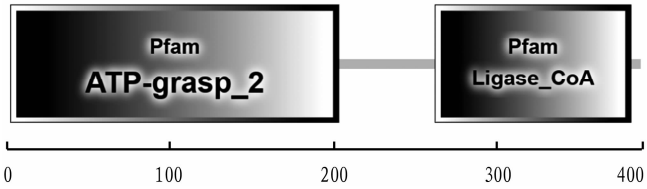


Fig. 5 Functional domain 1 of SucC

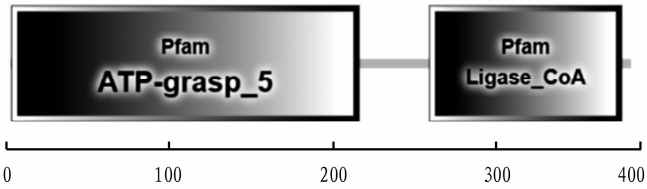
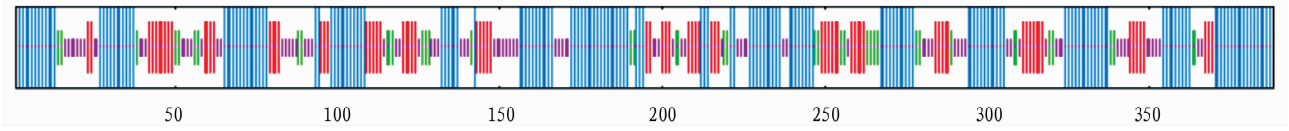


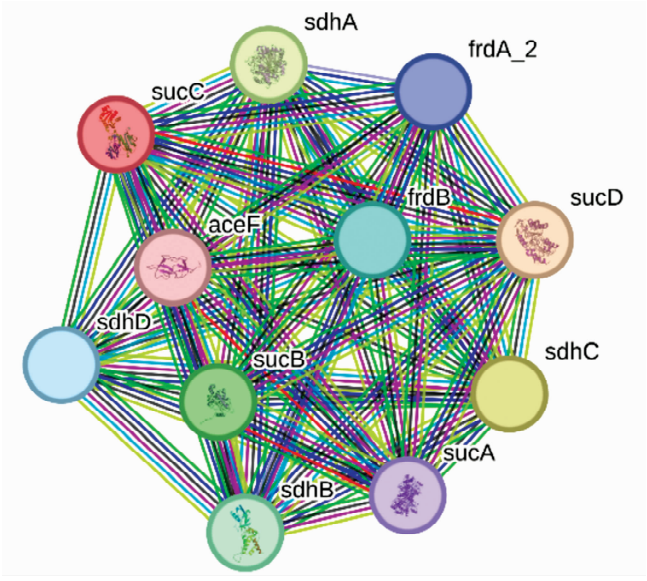
Fig. 6 Functional domain 2 of SucC



NOTE Blue: α-helix; purple: random coil; red: extended strand.

Fig. 7 Secondary structure prediction of SucC

The SucC protein structure was predicted by homology modeling. SucC amino acid sequence was uploaded in SWISS-MODEL program, and homologous protein with high similarity was selected as template (template: Q7MMN3.1.A) and compared to obtain SucC tertiary structure model with 96.91% similarity (Fig. 8), indicating that the protein structure predicted by this method was more accurate and could be used as a theoretical basis for subsequent experiments.



NOTE Template: Q7MMN3.1.A; similarity: 96.91%.

Fig. 8 Tertiary structure prediction of SucC

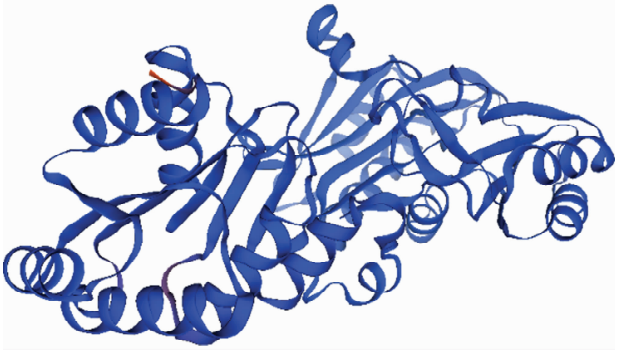


Fig. 9 PPI of SucC

3.6 SucC protein-protein interaction (PPI) In the PPI, the proteins adjacent to SucC protein are SucA, SucB, SucD, SdhD, SdhB, SdhA, SdhC, AceF, FfdA-2 and FfdB (Fig. 9), indicating that there may be potential interactions between them.

4 Conclusions and discussion

4.1 Conclusions The *sucC* gene of *Vibrio alginolyticus* HY9901 was 1 167 bp in length, encoding 389 amino acids, which were stable and non-hydrophilic. The theoretical pI value was 4.90, and the predicted molecular weight was 41 528.45 Da. There was no signal peptide and transmembrane domain. The subcellular localization prediction showed that the protein might be located in the cytoplasm. The amino acid sequence contained several sites, of which there were 8 N-terminal myristoylation sites, 6 casein kinase II phosphorylation sites, 5 microbody C-terminal target signal sites, one protein kinase C phosphorylation site, one pentenyl binding site and one endoplasmic reticulum signal peptide site; there were multiple modification sites, wherein 4 lactic acid modification sites were overlapped with ubiquitination modification sites. Besides, there were three functional domains in Pfam SucC, and its secondary structure was mainly composed of α-helix, random coil and a small amount of extended strands. The similarity of its tertiary structure model to the template Q7MMN3.1.a was 96.91%. The amino acid sequence of SucC of *V. alginolyticus* and *V. parahaemolyticus* has the highest homology of 99%, indicating that they are the closest in evolutionary relationship. In the PPI, SucA, SucB, SucD, SdhD, SdhB, SdhA, SdhC, AceF, FrdA-2, and FrdB were adjacent to the SucC protein. The results of this study will lay a foundation for the study of drug resistance mechanism of *V. alginolyticus*.

4.2 Discussion Based on the amino acid sequence of *sucC* gene of *V. alginolyticus* strain HY9901, we predicted the physical and chemical properties, functional domains, secondary and tertiary structures, homology, and sucC PPIs and analyzed the results, and explained the possible role of SucC protein. At present, the structure and function of *sucC* gene have been widely studied in prokaryotes, especially in *E. coli*. In *E. coli*, succinyl-CoA synthetase (SCS) is composed of two subunits, α and β, which form a tetrameric quaternary structure encoding the *E. coli* sucD and

sucC genes, respectively, and is part of the larger operon that drives the transcription of multiple TCA cycle proteins[26-27]. In PPI prediction results, SucC and SucD were adjacent proteins, which also confirmed this result. The *sucC* gene plays an important role in the TCA cycle by promoting the conversion of succinyl-CoA to succinate, which is a catalytic step coupled to substrate-level phosphorylation of GDP or ADP alone[28-29]. Shruti Kashyap *et al.*[30] believed that the downregulation of the *sucC* gene reflected reduced respiration, which may have reduced PMF and subsequently ATP production, leading to tobramycin-induced cell death. Feng Xu *et al.*[31] also found that SucC protein is the β subunit of succinyl-CoA synthetase, which can catalyze succinyl-CoA to succinate. These results are consistent with our results in the functional domain. Inactivation of succinyl-CoA synthetase significantly triggers sharp changes in carbon metabolic flux, thereby enhancing erythromycin production in E3Δ *sucC*. Campbell *et al.*[32] found that *sucC* inhibited autolytic activity and increased resistance of *Staphylococcus aureus* to β-lactam antibiotics by disrupting cell wall phenotype, while mutations in *sucC* and *sucD* led to the accumulation of succinyl-CoA, which in turn disrupted lysine succinylation in the proteome, and then affect the drug resistance of bacteria. Therefore, we speculate that SucC and SucD, as neighboring proteins, may also affect bacterial resistance by disrupting lysine succinylation. In addition, in *E. coli*, *ubiF* and *sucB* deletion mutants have been shown to reduce the durable survival of ampicillin and gentamicin and to increase susceptibility to different antibiotics[33]. In the prediction results of PPI, SucC and SucB, as neighboring proteins, may also have a certain relationship. Therefore, *sucC* may also act as an important gene in *V. alginolyticus* to regulate bacterial resistance to different antibiotics. We can further study the role and mechanism of *sucC* gene in transcriptional regulation by knocking out *sucC* gene in *V. alginolyticus* and comparing with wild strains. Post-translational modifications (PTMs), such as phosphorylation, ubiquitination, methylation, *etc.*, play an important role in the physiological and biochemical processes of various cells. When these modifications interact or combine together, the effect is called post-translational modification crosstalk[34-35]. Extensive studies have shown that different PTMs on proteins can interact or work together to perform specific biological functions to initiate or inhibit downstream signaling. Many crosstalks are classified primarily by two PTM interactions, but there are actually three or more modifiers involved in these processes[36]. In addition, the potential mechanism of PTM crosstalk in protein interaction remains to be studied. In the prediction of SucC amino acid sequence modification sites, we found that there was overlap between its lactic acid modification and ubiquitination modification sites, which may lead to crosstalk. This may provide abundant promising targets for the prevention and treatment of *V. alginolyticus*. In recent years, although the biological effects of lactic acid modification and ubiquitination modification have been studied, there are few studies on the crosstalk and interaction between them, which need to be further explored. Yao Rong *et al.*[37] mentioned that some specific enzymes such as prolyl hydroxylase 2 (PDH2) can mediate the degradation of ubiquitination modification, and lactic acid can participate in the regulation of tumor cell growth and related angiogenesis by inhibiting this degradation process and inhibiting the activity of PDH2. In *V. alginolyticus*, there may also be a relationship between lactic acid modification and ubiquitination modification, so it is necessary to explore the potential mechanism between them in the future.

References

[1] ALY SM, ELATTA MA, ELBANNA NI, *et al.* Comprehensive analysis of *V. alginolyticus*: Environmental risk factors in the cultured *Gilthead seabream* (*Sparus aurata*) under seasonal fluctuations and water parameter alterations[J]. Journal of Fish Disease, 2023, 46(12): 1425-1437.

[2] XUE SX, SUN JS. Detection of pathogenic bacterial (*Edwardsiella tarda* and *Vibrio alginolyticus*) associated with swollen abdomen of cultured turbot (*Scophthalmus maximus*) and flounder (*Paralichthys olivaceus*) by nested-PCR[J]. Acta Hydrobiologica Sinica, 2008(6): 856-860. (in Chinese).

[3] WEI S, WANG TJ, LONG Y, *et al.* Multiplex enrichment quantitative PCR Assays for the detection of *Vibrio alginolyticus*, *Vibrio parahaemolyticus*, *Vibrio vulnificus* and *Vibrio cholerae*[J]. Scientia Agricultura Sinica, 2016(49): 4619-4627. (in Chinese).

[4] WU HB, PAN JP. Purification and characterization of the exotoxin VM-PM produced by *Vibrio mimicus*[J]. Oceanologia Et Limnologia Sinica, 2002(1): 83-89. (in Chinese).

[5] LI YQ, JIAN JC, WU ZH. Pathogen of Vibriosis of *Lutjanus sanguineus*[J]. Journal of Zhanjiang Ocean University, 2004(6): 1-5. (in Chinese).

[6] HUANG ZJ, HE JG. Isolation, identification and pathogenicity of the bacterial pathogen in *Epinephlus fario*[J]. Acta Scientiarum Naturalium Universitatis Sunyatseni, 2002(5): 64-67. (in Chinese).

[7] WONG KC, BROWN AM, LUSCOMBE GM, *et al.* Antibiotic use for Vibrio infections: important insights from surveillance data[J]. BMC Infectious Diseases, 2015, 15(1): 226.

[8] XIONG X, BROMLEY EHC, OELSCHLAEGER P, *et al.* Structural insights into quinolone antibiotic resistance mediated by pentapeptide repeat proteins: Conserved surface loops direct the activity of a Qnr protein from a Gram-negative bacterium[J]. Nucleic Acids Research, 2011, 39(9): 3917-3927.

[9] DARBY EM, TRAMPARI E, SIASAT P, *et al.* Molecular mechanisms of antibiotic resistance revisited[J]. Nature Reviews Microbiology, 2023, 21(5): 280-295.

[10] PENG B, SU YB, LI H, *et al.* Exogenous alanine and/or glucose plus kanamycin kills antibiotic-resistant bacteria[J]. Cell Metabolism, 2015, 21(2): 249-262.

[11] CHENG ZX, YANG MJ, PENG B, *et al.* The depressed central carbon and energy metabolisms is associated to the acquisition of levofloxacin resistance in *Vibrio alginolyticus*[J]. Journal of Proteomics, 2018(181): 83-91.

[12] HUANG J, FRASER ME. Structural basis for the binding of succinate to succinyl-CoA synthetase[J]. Acta Crystallographica Section D, 2016, 72(8): 912-921.

[13] FRASER ME, JAMES MNG, BRIDGER WA, *et al.* A detailed structural description of *Escherichia coli* succinyl-CoA synthetase11 edited by D. Rees[J]. Journal of Molecular Biology, 1999, 285(4): 1633-1653.

[14] ZENG FY. Effect of lysine succinylation of PEPCK on drug resistance of

Vibrio alginolyticus [D]. Zhanjiang: Guangdong Ocean University, 2022. (in Chinese).
[15] CAI SH, WU ZH, JIAN JC, *et al.* Cloning and expression of the gene encoding an extracellular alkaline serine protease from *Vibrio alginolyticus* strain HY9901, the causative agent of vibriosis in *Lutjanus erythopterus* (Bloch)[J]. Journal of Fishe Diseases, 2007, 30(8): 493 – 500.
[16] SARIPELLA GV, SONNHAMMER ELL, FORSLUND K. Benchmarking the next generation of homology inference tools[J]. Bioinformatics, 2016, 32(17): 2636 – 2641.
[17] GASTEIGER E, GATTIKER A, HOOGLAND C, *et al.* ExPASy: The proteomics server for in-depth protein knowledge and analysis[J]. Nucleic Acids Research, 2003, 31(13): 3784 – 3788.
[18] NIELSEN H. Predicting Secretory Proteins with SignalP[M]//KIHARA D. Protein Function Prediction: Methods and Protocols. New York: Springer New York. 2017: 59 – 73.
[19] SONNHAMMER EL, VON HEIJNE G, KROGH A. A hidden Markov model for predicting transmembrane helices in protein sequences[J]. Proceedings International Conference on Intelligent Systems for Molecular Biology, 1998(6): 175 – 182.
[20] WANG D, LIU D, YUCHI J, *et al.* MusiteDeep: A deep-learning based webserver for protein post-translational modification site prediction and visualization[J]. Nucleic Acids Research, 2020, 48(W1): W140 – W146.
[21] JIANG P, NING W, SHI Y, *et al.* FSL-Kla: A few-shot learning-based multi-feature hybrid system for lactylation site prediction[J]. Computational and Structural Biotechnology Journal, 2021-08-013
[22] TAMURA K, PETERSON D, PETERSON N, *et al.* MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods[J]. Molecular Biology and Evolution, 2011, 28(10): 2731 – 2739.
[23] SCHWEDE, TORSTEN, KOPP, *et al.* SWISS-MODEL: An automated protein homology-modeling server[J]. Nucleic Acids Research, 2003.
[24] SZKLARCZYK D, FRANCESCHINI A, KUHN M, *et al.* The STRING database in 2011: Functional interaction networks of proteins, globally integrated and scored[J]. Nucleic Acids Research, 2010, 39(suppl_1): D561 – D8.
[25] WANG D, LIANG Y, XU D. Capsule network for protein post-translational modification site prediction[J]. Bioinformatics, 2018, 35(14): 2386 – 2394.
[26] BUCK D, SPENCER ME, GUEST JR. Primary structure of the succinyl-CoA synthetase of *Escherichia coli*[J]. Biochemistry, 1985, 24(22): 6245 – 6252.
[27] FRASER ME, JAMES MNG, BRIDGER WA, *et al.* A detailed structural description of *Escherichia coli* succinyl-CoA synthetase[J]. Journal of Molecular Biology, 1999, 285(4):1633 – 1653.
[28] NISHIMURA JS. Succinyl-CoA synthetase structure-function relationships and other considerations[M]. Advances in Enzymology and Related Areas of Molecular Biology. 1986: 141 – 72.
[29] BRIDGER WA. 18. Succinyl-Coa Synthetase[M]//BOYER P D. The Enzymes. Academic Press. 1974: 581 – 606.
[30] KASHYAP S, SHARMA P, CAPALASH N. Tobramycin stress induced differential gene expression in *Acinetobacter baumannii*[J]. Current Microbiology, 2022, 79(3): 88.
[31] XU F, KE X, HONG M, *et al.* Exploring the metabolic fate of propanol in industrial erythromycin-producing strain via 13C labeling experiments and enhancement of erythromycin production by rational metabolic engineering of Saccharopolyspora erythraea[J]. Biochemical and Biophysical Research Communications, 2021(542): 73 – 79.
[32] CAMPBELL C, FINGLETON C, ZEDEN MS, *et al.* Accumulation of succinyl coenzyme A perturbs the methicillin-resistant *Staphylococcus aureus* (MRSA) succinylome and is associated with increased susceptibility to beta-lactam antibiotics[J]. 2021, 12(3): e0053021
[33] MA C, SIM S, SHI W, *et al.* Energy production genes sucB and ubiF are involved in persister survival and tolerance to multiple antibiotics and stresses in *Escherichia coli*[J]. FEMS Microbiology Letters, 2010, 303(1): 33 – 40.
[34] MACEK B, FORCHHAMMER K, HARDOUIN J, *et al.* Protein post-translational modifications in bacteria[J]. Nature reviews Microbiology, 2019, 17(11): 651 – 664.
[35] HUNTER T. The age of crosstalk: Phosphorylation, ubiquitination, and beyond[J]. Molecular cell, 2007, 28(5): 730 – 738.
[36] WU Z, HUANG R, YUAN L. Crosstalk of intracellular post-translational modifications in cancer[J]. Archives of Biochemistry and Biophysics, 2019(676): 108138.
[37] RONG Y, DONG F, ZHANG G, *et al.* The crosstalking of lactate-Histone lactylation and tumor[J]. 2023, 17(5): 2200102.

(From page 31)

[4] KIM DJ, LEE MH, LIU K, *et al.* Herbacetin suppresses cutaneous squamous cell carcinoma and melanoma cell growth by targeting AKT and ODC[J]. Carcinogenesis, 2017, 38(11): 1136 – 1146.
[5] LI L, FAN P, CHOU H, *et al.* Herbacetin suppressed MMP9 mediated angiogenesis of malignant melanoma through blocking EGFR-ERK/AKT signaling pathway[J]. Biochimie, 2019(162): 198 – 207.
[6] HYUGA S, HYUGA M, YOSHIMURA M, *et al.* Herbacetin, a constituent of ephedrae herba, suppresses the HGF-induced motility of human breast cancer MDA-MB-231 cells by inhibiting c-Met and Akt phosphorylation[J]. Planta Medica, 2013, 79(16): 1525 – 1530.
[7] KIM DJ, ROH E, LEE MH, *et al.* Herbacetin is a novel allosteric inhibitor of ornithine decarboxylase with antitumor activity[J]. Cancer Research, 2016, 76(5): 1146 – 1157.
[8] QIAO Y, LIU XB. The effect of herbacetin on scavenging free radicals and inhibiting protein oxidation *in vitro*[J]. Food Science, 2013, 34(17): 106 – 110. (in Chinese).
[9] WANG Y, WANG N, LIU YY *et al.* Effects of herbacetin on hepatic steatosis and intrahepatic oxidative stress in rats with non-alcoholic steatohepatitis[J]. Journal of Practical Hepatology, 2020, 23(5): 626 – 629. (in Chinese).
[10] LI L, SAPKOTA M, KIM SW, *et al.* Herbacetin inhibits inducible nitric oxide synthase via JNK and nuclear factor-κB in LPS-stimulated RAW264.7 cells[J]. European Journal of Pharmacology, 2015(765): 115 – 23.
[11] IJAZ MU, MUSTAFA S, BATOOL R, *et al.* Ameliorative effect of herbacetin against cyclophosphamide-induced nephrotoxicity in rats via attenuation of oxidative stress, inflammation, apoptosis and mitochondrial dysfunction[J]. Human & Experimental Toxicology, 2022: 41.