Proteomic Analysis of *Stichopus japonicus* and Evaluation of the Antiaging Properties of Its Peptide

Le WANG[1#], Jiao CHEN[2#], Jiali WENG[2], Jinxing FU[2], Wenqin SU[2], Bingmiao GAO[2*], Qin CHEN[3*]

1. Department of Food and Pharmacy, Hainan Health Management College, Chengmai 571999, China; 2. School of Pharmacy, Hainan Medical University, Haikou 571199, China; 3. Hainan Open University, Haikou 570208, China

Abstract The sea cucumber *Stichopus japonicus* is one of the "Eight Treasures of Seafood" and contains a number of bioactive components involved in multiple physiological and pharmacological functions. Proteins and peptides are generally considered to be responsible for these beneficial properties. In this study, a total of 3 478 proteins and 17 390 peptides were identified in *Stichopus japonicus* by proteomics methods. Among them, 4 proteins were involved in 8 metabolic pathways, especially oxidative phosphorylation and cell senescence. Subsequently, lifespan assay and oxidative stress test were performed to investigate the peptides prepared from sea cucumber protein hydrolyzate using the aging model of *Caenorhabditis elegans*. The results of the anti-aging experiment demonstrated that high-dose peptides significantly prolonged the lifespan of nematodes (30.50%), and improved their capacity to inhibit oxidative stress. The results provide evidence supporting the development of bioactive proteins and peptides derived from *Stichopus japonicus* as functional foods and lay the foundation for the research of an anti-aging drug.

Key words *Stichopus japonicus*; Proteomics; Protein extraction; *Caenorhabditis elegans*; Anti-aging

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Stichopus japonicus, commonly known as the "king of sea cucumbers", belongs to the family Stichopodidae, order Aspidochirota, class Holothuroidea, and phylum Echinodermata. It is usually found in shallow sea areas with a water depth ranging between 20 - 30 m, and has long been exploited in many countries for its abundant nutritional value and potential economic interest, especially in China, Russia, Japan, and Korea[1]. As one of the "Eight Treasures of Seafood", *S. japonicus* has high protein and low lipid contents, and is free from cholesterol, possessing a number of bioactive components involved in multiple physiological and pharmacological functions[2-3]. Modern pharmacological studies have revealed the various pharmacological properties of the peptides of *S. japonicus*, including anti-aging, anti-fatigue, anti-oxidation, anti-coagulation, anti-hyperlipidemia, anti-hypertension, anti-obesity, and immune regulation[4-5].

Proteomics is one of the hotspots in life science and research techniques that have been rapidly developed[6]. The combination of proteomics and bioinformatics has given rise to an influential tool to investigate the mechanisms of change at the protein level that affect quality attributes by comparing identified peptides or proteins to a specific database[7]. Currently, it has been successfully applied to study the geographic variation and the physiological response of *S. japonicus* in various situations[8-9]. Extensive literature indicates that sea cucumber peptides released by enzymatic hydrolysis have potential application value in enhancing antioxidant and immune functions, as well as delaying senescence[10-11]. However, proteomics has not been applied in the bioinformatics analysis of sea cucumber peptides from *S. japonicus*.

Aging is an inevitable and degenerative process of the body's physical and psychological adaptability to external stress[12]. More than 70 percent of the human lifespan is determined by external environmental factors such as lifestyle, medical level, natural, and social environments[13]. *Caenorhabditis elegans* is a classical model organism for research related to aging, age-related diseases, and mechanisms of longevity due to its short life span, easy laboratory handling, and amenability to genetic manipulation[10,14]. So far, proteomic studies and *in vivo* experiments on the anti-aging peptides prepared by enzymatic hydrolysis from *S. japonicus* proteins have been rather limited.

This study aimed to investigate and preliminarily identify the proteins and peptides of *S. japonicus* by using a combination of bioinformatics and proteomics. Meanwhile, the effects of these peptides on the lifespan and oxidative stress response of *C. elegans* were observed, laying a scientific foundation for research and development of proteins and peptides from *S. japonicus*.

Materials and Methods

Materials and chemicals

The alkaline proteases (01-186) were provided by Aoboxing Biotechnology Co., Ltd. (Beijing, China). Protein Marker was obtained from Jiangsu Churui Biotechnology Co., Ltd. In addition,

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Le WANG (1987 -), female, P. R. China, master, lecturer, devoted to research about marine functional food.

Jiao CHEN (1992 -), female, P. R. China, master, experimentalist, devoted to research about the marine bioactive peptides.

#These authors contribute equally to this work.

* Corresponding author.

Coomassie Brilliant Blue R250 and Coomassie Blue Fast Stain Kit (PH0351) were provided by Phygene Life Sciences Co., Ltd (Fuzhou, China). The BCA Protein Assay Kit and SDS-PAGE were obtained from Biosharp Life Sciences Co., Ltd., while the RIPA Lysis Buffer was purchased from Beijing Applygen Technologies Inc. Peptone was obtained from Beijing BioDee Biotechnology Co., Ltd. All the other reagents used were of analytical grade.

Total protein extraction

Fresh sea cucumber (*S. japonicus*) was purchased from a local aquatic market in Haikou, China. RIPA lysis buffer was added to the tissue samples, followed by ultrasonic lysis in an ice bath to extract total proteins. The homogenized samples were kept at 4 ℃ and the extraction was continued for 10 min, and then, the samples were centrifuged (12 000 rpm, 4 ℃, 15 min). Finally, the supernatant was collected and stored at −80 ℃. The protein powders of *S. japonicas* were obtained after freeze-drying. These powders were resolved and the total protein was identified by SDS-PAGE. In addition, the protein contents were evaluated with a BCA kit following the manufacturer's instructions.

Trypsin digestion and LC-MS/MS analysis

For digestion, trypsin was added to the protein extracts at a trypsin: protein ratio of 1 ∶ 50 and incubated overnight at 37 ℃. Tryptic peptides were obtained by centrifugation, purification, and other steps. Subsequently, LC-MS/MS analysis was performed by using an EASY-nLC 1200 UHPLC system coupled to a Q Exactive HF-X mass spectrometer (Thermo Scientific, USA) with a Nanospray Flex ESI source.

Database search, protein identification and quantification

Raw data files were searched using Proteome Discoverer (version 2.4) software embedded with Sequest HT search engine against the *S. japonicus* proteome database. MS spectra lists were searched against their species-level UniProt FASTA databases. The mass tolerance was set to 10 ppm for precursor ions and 0.02 Da for the fragment ions. Unique peptide and Razor peptide were used for quantification of the identified protein and normalization of all peptides. All other Settings were left at the default values.

Bioinformatics analysis

Gene Ontology (GO) and the Kyoto Encyclopedia of Genes and Genomes (KEGG) were used to analyze the identified proteins. According to GO analysis, these identified proteins were classified into three groups: biological processes (BP), cellular components (CC), and molecular functions (MF)[15-16]. Meanwhile, the protein pathways and metabolic pathways were annotated based on the KEGG annotation[17].

Preparation of *S. japonicus* peptide

The extraction of *S. japonicus* peptide was carried out following the methods of a previous study[18]. An appropriate amount of protein powder of sea cucumber was added to deionized water at a ratio of 1∶10 (w/v), and hydrolyzed with alkaline protease (4%). Then, the resultant mixture was adjusted to pH 8.0, and enzymatic hydrolysis was performed at 55 ℃ for 3 h. The enzymatic reaction was stopped by heating at 95 ℃ for 5 min and centrifuged at 4 000 r/min for 10 min. The supernatant was then collected and freeze-dried as peptide powder of *S. japonicus*.

Lifespan analysis of *C. elegans*

The growth conditions and synchronization of *C. elegans* were performed as described in previous studies[10,19]. Synchronized L4 larvae from each group were transferred randomly to nematode growth medium (NGM) plates and fed with OP50 mixed with *S. japonicus* peptide at different concentrations (0, 0.125, 0.250, and 0.500 mg/ml). Triplicate plates were used for each group (30 per plate) at 20 ℃ for each experiment. During the lifespan assay, the NGM medium was replaced every t3 d, while the number of live and dead nematodes was scored daily from the first day until all died. The survival curves diagram was drawn and analyzed statistically.

Response to oxidative stress

For the oxidative stress assay, synchronized L4 larvae (3 plates per group and 30 worms per plate) were pretreated with *S. japonicus* peptide (0, 0.125, 0.250, and 0.500 mg/ml) at 20 ℃ for 72 h. Subsequently, each group's larvae were transferred to NGM media containing 30% hydrogen peroxide (10 μl of 30% hydrogen peroxide per 10 ml of NGM). The number of surviving and dead worms was counted every 1 h until all worms died.

Data analysis

Data processing and statistical analyses were performed using GraphPad Prism 8 by student's *t*-test. In this study, $P < 0.05$ was considered statistically significant. The peak area of specific individual protein was calculated by relative peak intensities and the average protein abundance between groups was also calculated.

Results and Analysis

Extraction of protein and its SDS-PAGE analysis

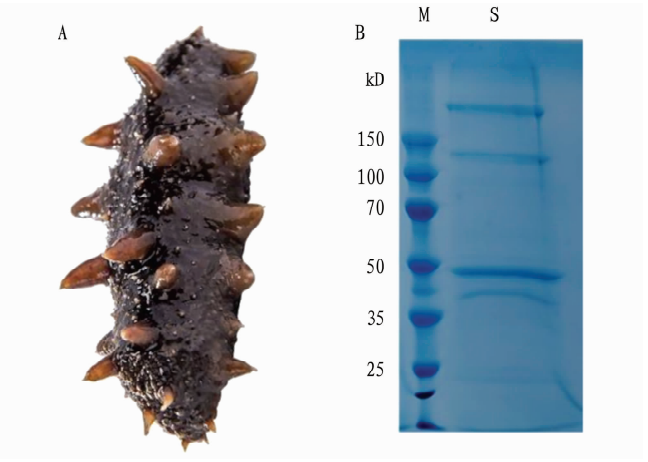
The obtained *S. japonicus* was identified by experts, as shown in Fig. 1A. The concentration of total peptide extracted from *S. japonicus* was 0.27 mg/ml. The SDS-PAGE results are displayed in Fig. 1B, showing the molecular weight of all proteins of *S. japonicus*. Among them, four electrophoretic bands with clear boundaries were above 100 kDa, with some proteins around 20 to 50 kDa.

Identification of proteins and peptides by quantitative proteomics analysis

As shown in Fig. 2, a total of 3 478 proteins and 17 390 peptides were identified using LC-MS/MS proteomic analyses after trypsin hydrolysis. The results indicated that the majority of the identified proteins of *S. japonicus* had a molecular weight between 20 and 60 kDa. Moreover, the protein enzymolysis peptide with 7 − 13 amino acids was the most abundant, which laid a material foundation for further research on the pharmacological activity of *S. japonicus* peptides.

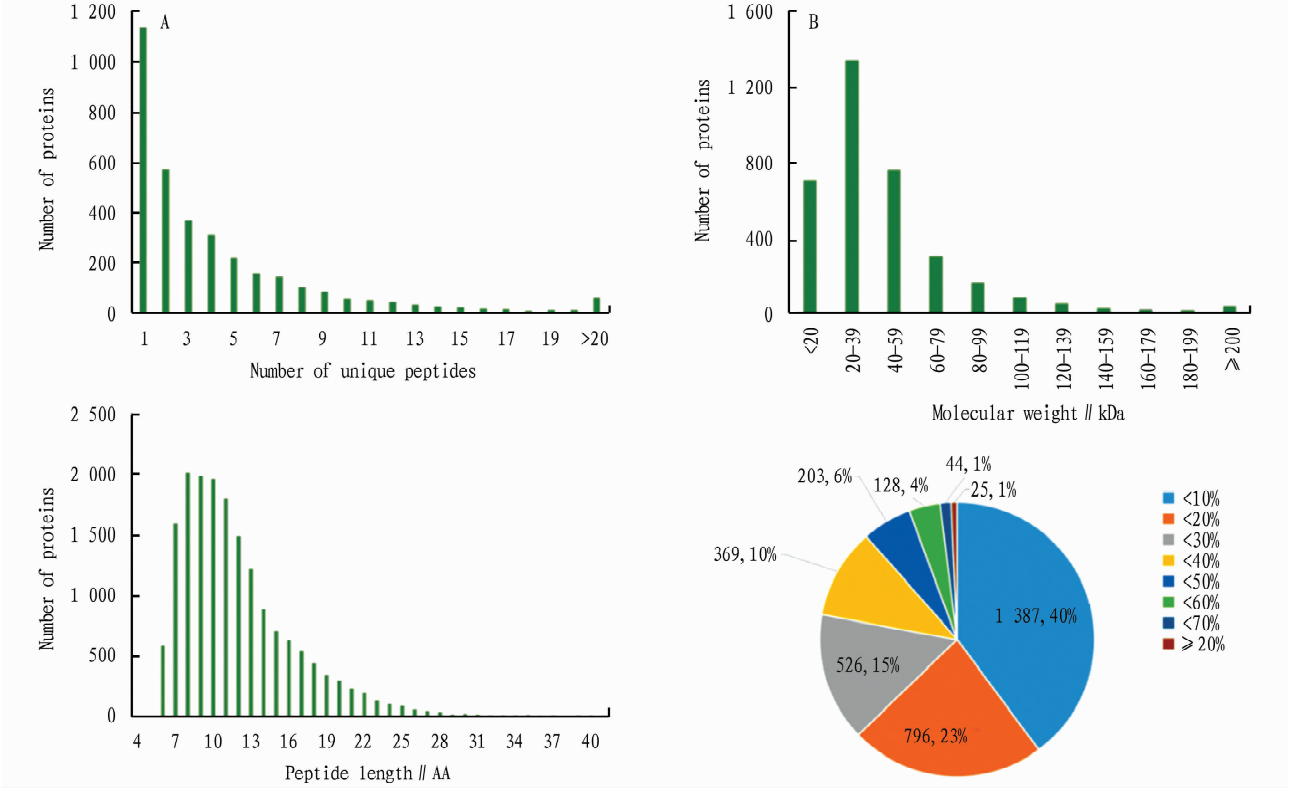
GO analysis

Based on gene ontology (GO) annotation, all the identified proteins were further categorized into biological processes (BP), cellular components (CC), and molecular functions (MF), as shown in Fig. 3. The matched BP of identified proteins was 18 383, CC was 11 797, and MF was 12 959. Among the BP terms, "metabolic process" (5.7%), "organic substance metabolic process" (5.2%), "organonitrogen compound metabolic process" (3.7%), "protein metabolic process" (2.7%), and "cellular protein metabolic process" (2.2%) were considerably more predominant in the identified proteins compared with other proteins. Among the GO CC terms, "cell" (7.5%), "cell part" (7.5%), "intracellular" (7.2%), "intracellular part" (7.2%) and "organelle" (5.5%) accounted for a larger percentage. With respect to MF terms, "binding" (9.2%), "catalytic activity" (9.1%), "ion binding" (5.8%), "heterocyclic compound binding" (5.7%), and "organic cyclic compound binding" (5.7%) were considerably more predominant in the identified proteins compared with other proteins.



A. A picture of *S. japonicus*; B. SDS-PAGE analysis of its proteins; M: Protein marker; S: Protein sample of *S. japonicus*.

Fig. 1 SDS-PAGE results



A. Numbers of identified proteins and peptides; B. Molecular weight distributions of proteins in *S. japonicus*; C. Distribution of peptide chain length obtained by enzymatic hydrolysis of proteins; D. Distribution of peptide coverage of the identified proteins.

Fig. 2 Identification and distribution of proteins and peptides in *S. japonicus*

KEGG pathway analysis

The KEGG annotation results of proteins of *S. japonicus* are shown in Table 1 and Fig. 4, revealing the types of metabolic pathways of proteins in *S. japonicus*. KEGG pathway enrichment showed that four identified proteins (COX1, ND4, hsc70, and rbbp4) were mainly involved in oxidative phosphorylation, cardiac muscle contraction, spliceosome, protein processing in the endoplasmic reticulum, cellular senescence, endocytosis, metabolic pathways, and MAPK signaling pathway (Table 1). As illustrated in Fig. 4, the significantly enriched proteins COX1 and ND4 were involved in the oxidative phosphorylation and metabolic pathways. In particular, the expression levels of COX1 and ND4 were both up-regulated in oxidative phosphorylation related to anti-oxidation, indicating that *S. japonicus* exerts a certain pharmacological action against aging.

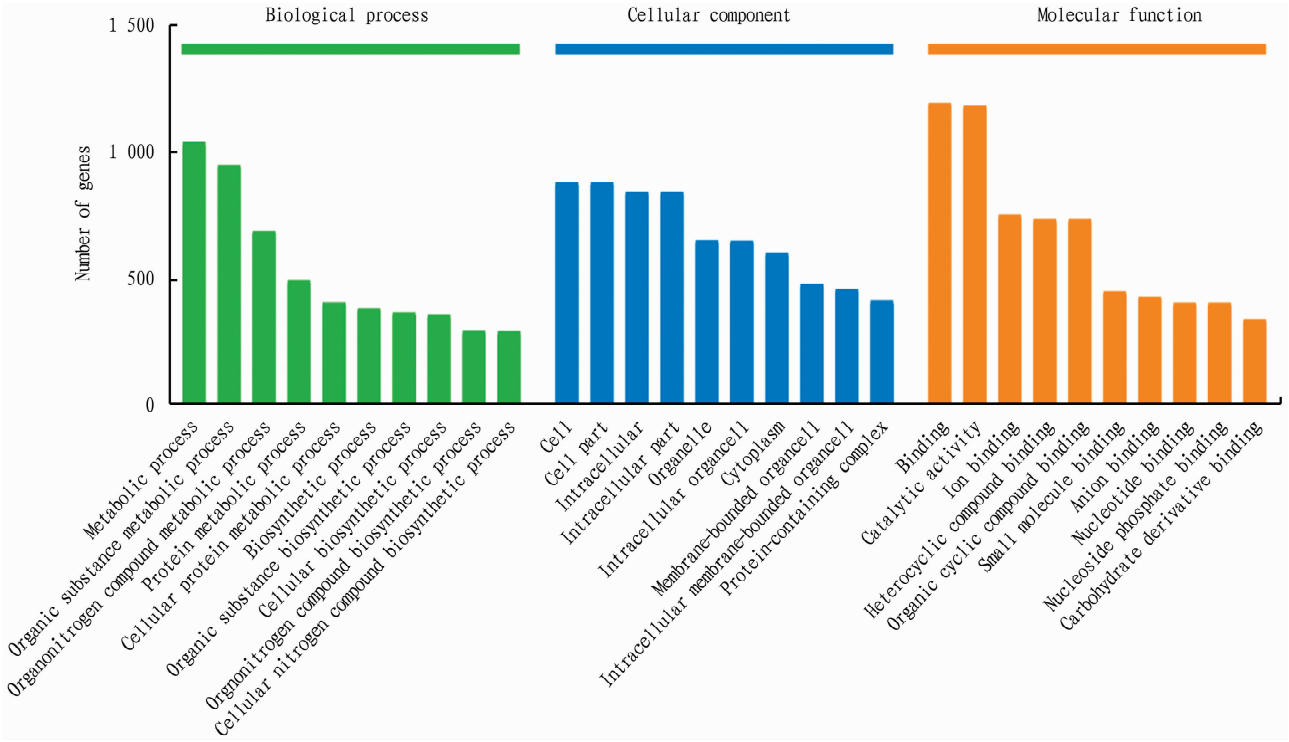


Fig. 3 GO analysis of all the identified and quantified proteins of *S. japonicus*

Table 1 KEGG pathway enrichment analysis of identified proteins of *S. japonicus*

ID	Description	GeneRatio	BgRatio	Genes	Count
dre00190	Oxidative phosphorylation	2/4	140/5928	COX1||ND4	2
dre04260	Cardiac muscle contraction	1/4	98/5928	COX1	1
dre03040	Spliceosome	1/4	134/5928	hsc70	1
dre04141	Protein processing in the endoplasmic reticulum	1/4	181/5928	hsc70	1
dre04218	Cellular senescence	1/4	187/5928	rbbp4	1
dre04144	Endocytosis	1/4	311/5928	hsc70	1
dre01100	Metabolic pathways	2/4	1364/5928	COX1||ND4	2
dre04010	MAPK signaling pathway	1/4	373/5928	hsc70	1

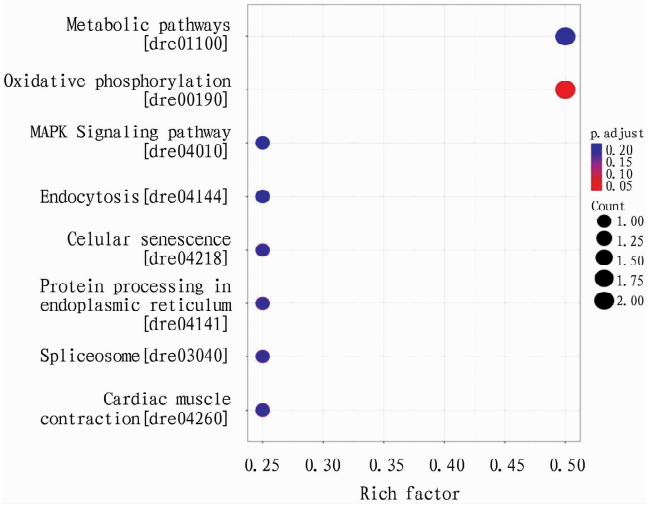


Fig. 4 KEGG enrichment analyses of differentially expressed proteins in *S. japonicus*

Effects of *S. japonicus* peptide on the lifespan of *C. elegans*

To determine the influence of the *S. japonicus* peptide on longevity, *C. elegans* at the L4 stage were treated with extracts at concentrations ranging from 0 to 0.500 mg/ml in standard NGM. The low dose (0.125 mg/ml), medium dose (0.250 mg/ml), and high dose (0.500 mg/ml) groups showed mean lifespans of (14.93 ± 0.21), (16.87 ± 0.25), and (18.40 ± 0.10) d, accounting for a percentage increased lifespan of 5.89, 19.65 and 30.50, respectively (Table 2). Meanwhile, the maximum lifespan of the three groups was increased by 4.63%, 14.70%, and 20.46 % compared to the control group. As shown in Fig. 5, the lifespan-prolonging effect on *C. elegans* gradually increased as the concentration of *S. japonicus* peptides was increased, showing a dose-response relationship. These results revealed that *S. japonicus* peptides exhibited an anti-aging effect in the *C. elegans* model.

Table 2 Effects of *S. japonicus* peptide on the lifespans of worms

Group	Mean lifespan ± SD // d	Increase of lifespan // %	Maximum lifespan // d	Increase of lifespan // %
Control	14.100.56	–	18.570.40	–
Low dose (0.125 mg/ml)	14.930.21	5.89	19.430.15	4.63
Medium dose (0.250 mg/ml)	16.870.25 *	19.65	21.300.21 *	14.70
High dose (0.500 mg/ml)	18.400.10 * *	30.50	22.370.31 *	20.46

Significant difference in lifespan between the treated group and the untreated control group ($^{*}P<0.05$, $^{**}P<0.01$).

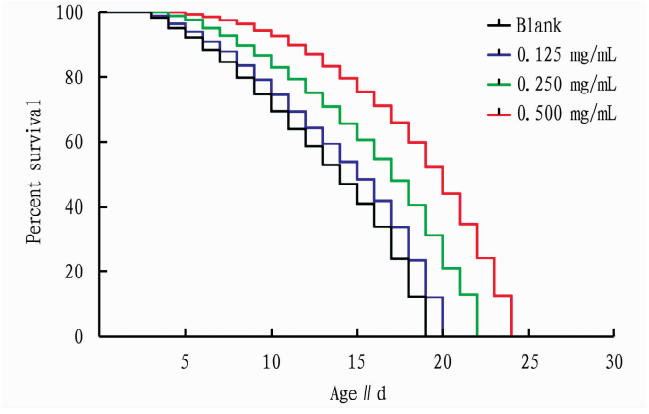


Fig. 5 Survival curves of *C. elegans* treated with different concentrations of *S. japonicus* peptides

Effects of *S. japonicus* peptide on resistance to oxidative stress by *C. elegans*

Hydrogen peroxide was used in the oxidative stress assay. As illustrated in Table 3, 0.250 and 0.500 mg/ml of *S. japonicus* peptides significantly increased resistance to oxidative stress ($P<0.05$), while the low dose (0.125 mg/ml) group only slightly increased the resistance to the stressor compared to the control group. Moreover, when the nematodes were treated with *S. japonicus* peptides at the concentrations used (0.125 – 0.500 mg/ml), the lifespan was substantially extended in a dose-dependent manner compared to that of the nematodes exposed to hydrogen peroxide alone (Fig. 6), indicating that *S. japonicus* peptides could improve nematodes capacity for stress tolerance. The mean lifespans of low dose, medium dose, and high dose were increased by 11.00 %, 16.24 %, and 44.28 %, respectively, compared to the control group.

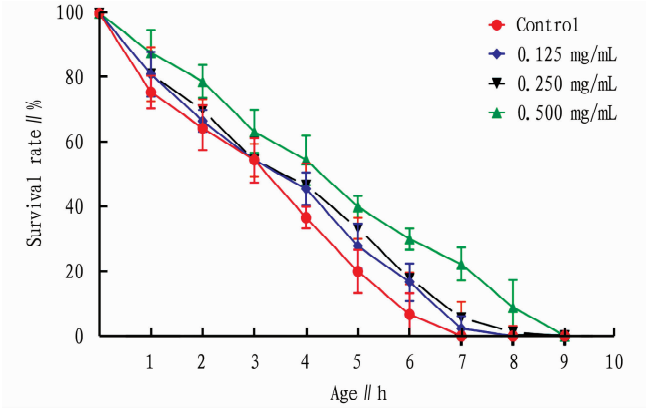


Fig. 6 The survival curves of *C. elegans* exposed to oxidative stress injury

Table 3 Lifespan-extension effects of *S. japonicus* peptide on the *C. elegans* with oxidative stress injury

Group	Mean lifespan ± SD // h	Increase of lifespan // %	Maximum lifespan // h
Control	2.71 ± 0.45	–	6.0
Low dose (0.125 mg/ml)	3.01 ± 0.43	11.00	6.3
Medium dose (0.250 mg/ml)	3.15 ± 0.37 *	16.24	7.0 *
High dose (0.500 mg/ml)	3.91 ± 0.46 * *	44.28	8.0 * *

Significant differences in lifespan between the treated group and the untreated control group were denoted by $^{*}P<0.05$ and $^{**}P<0.01$.

Discussion

The aging of the population has emerged as an increasingly serious problem in modern society, and researching research anti-aging health foods and medicines is of great social significance[13]. Exploring the anti-aging effects of sea cucumber peptides has an important academic significance and practical value for the development of anti-aging drugs. The results could delay senility and decrease the incidence of age-related diseases. *S. japonicus* is an echinoderm and is a precious food product containing various nutritional components and bioactive compounds, including saponins, fatty acids, mucopolysaccharides, flavonoids, and bioactive proteins (collagen, peptides, amino acids)[20-21].

In this work, a total of 3 478 proteins and 17 390 peptides were identified from the results of the proteomics analysis (Fig. 2). Meanwhile, the GO and KEGG analyses revealed that four significantly enriched proteins (hsc70, COX1, ND4, and rbbp4) were involved in eight metabolic pathways (Fig. 3 and Fig. 4). In particular, the COX1 and ND4 proteins were closely associated with oxidative phosphorylation. The current research shows that most age-related illnesses may impair oxidative phosphorylation within mitochondria[22-23]. Herein, the upregulated expression of proteins COX1 and ND4 involved in the metabolism of oxidative phosphorylation may be responsible for the anti-aging pharmacological action of *S. japonicas*. Moreover, the proteomics results also revealed that the *S. japonicus* extract obtained by protease hydrolysis was abundant in peptides with a length of 7 – 13 amino acids (Fig. 2). Some researchers argue that many bioactive peptides have a relatively short peptide residue length[24-25]. In the sea cucumber peptides studies, these peptides have demonstrated various biological functions such as anti-inflammation, alleviating oxidative stress, neuro-protection, lifetime extension, and so on[26-27]. For instance, previous research has suggested that several oligopeptides from sea cucumber hydrolysates exhibit significant anti-Aβ aggregation activity that may be applied in the treatment of Alzheimer's disease[28]. Another study revealed that peptides hydrolyzed from *Holothuria nobilis* exerted anti-diabetic effects in

high-fat-diet-induced diabetic rats by regulating the PI3K/Akt signaling pathway[29].

In this study, *C. elegans* was used to demonstrate whether the peptides derived from *S. japonicas* can prolong the lifespan of an organism and improve its response to oxidative stress. *C. elegans* is a commonly used model to investigate age-related diseases and mechanisms due to its short lifespan, high amount of progeny, and availability of mutants [30-31]. Age-related diseases have been consistently linked to increased oxidative stress, so anti-senility experiments are usually performed in addition to anti-oxidative tests[32-33]. In this study, the lifespan assay and oxidative stress test demonstrated that various concentrations of peptides released by enzymatic hydrolysis of *S. japonicas* significantly increased the lifespan of nematodes (Fig. 5), and improved their resistance to oxidative stress (Fig. 6). The results are consistent with a previous study demonstrating the anti-oxidant and anti-aging effects of peptides from *A. japonicus*[34]. Similarly, peptides derived from the sea cucumber *I. badionotus*[35] also exerted anti-aging effects and increased the antioxidant capacity in a *C. elegans* model. Moreover, *S. variegates* peptides also exerted anti-aging activity and anti-oxidative stress effects in fruit flies and mice by up-regulating Klotho expression[36]. So far, the mechanisms underlying the anti-aging effects of sea cucumber peptides have not been conclusively elucidated[27]. Previous studies indicate that peptides from sea cucumbers show strong scavenging of harmful free radicals (DPPH, superoxide anions, and hydroxyl radical), thereby protecting cells and tissues against oxidative damage[11, 37]. Additionally, peptides obtained by biological enzymatic hydrolysis from sea cucumber have various positive characteristics, such as easy digestion, excellent solubility, favorable emulsification, and good absorption[38]. Nevertheless, further experiments and bioinformatics analyses are warranted to investigate the underlying anti-aging mechanism.

Conclusion

In summary, the proteins and peptides of *S. japonicas* were preliminarily identified and quantified by using a combination of bioinformatics and proteomics. The anti-aging protein-related signal pathway was also elucidated by bioinformatics. Further experiments indicated that peptides obtained by enzymatic hydrolysis could effectively promote longevity and attenuate hydroperoxide-induced oxidative injury in the *C. elegans* model system, thereby verifying the anti-aging effect of *S. japonicas* peptides which were consistent with the predicted results obtained by bioinformatics. These results provide insights into the development of bioactive proteins and peptides derived from *S. japonicas* for applications in functional foods and provide a basis for the research of anti-aging drugs.

Acknowledgement

Wang Le and Chen Jiao carried out most of the experimental work and were responsible for drafting the manuscript. Chen Qin and Weng Jiali conducted the extraction experiments and analyzed the results. Su Wenqin and Fu Jinxing performed the anti-aging experiment with sea cucumber peptides. Gao Bingmiao and Chen Qin provided scientific and administrative oversight for the research and funding acquisition, as well as revised the manuscript. All authors helped in the preparation of the manuscript.

References

[1] ZHOU Y, YANG H, LIU S, *et al.* Feeding and growth on bivalve biodeposits by the deposit feeder *Stichopus japonicus* Selenka (Echinodermata: Holothuroidea) co-cultured in lantern nets [J]. Aquaculture, 2006 (256): 510-520.

[2] ZHANG M, LIU YX, WU ZX, *et al.* Analysis of texture properties and water-soluble fraction proteome of sea cucumber body wall with different boiling heating treatment[J]. Food chemistry, 2023(409): 135333.

[3] KHOTIMCHENKO Y. Pharmacological potential of sea cucumbers[J]. International journal of molecular sciences,2018(19): 1342.

[4] ZHOU X, WANG C, JIANG A. Antioxidant peptides isolated from sea cucumber *Stichopus Japonicus*[J]. European Food Research and Technology, 2011(234): 441-447.

[5] GONG PX, WANG BK, WU YC, *et al.* Release of antidiabetic peptides from *Stichopus japonicas* by simulated gastrointestinal digestion[J]. Food chemistry, 2020(315): 126273.

[6] WILKINS MR, PASQUALI C, APPEL RD, *et al.* From proteins to proteomes: large scale protein identification by two-dimensional electrophoresis and amino acid analysis[J]. Bio/technology (Nature Publishing Company),1996(14): 61-65.

[7] HUANG YZ, XIE YS, LI YX, *et al.* Quality assessment of variable collagen tissues of sea cucumber (*Stichopus japonicus*) body wall under different heat treatment durations by label-Free proteomics analysis[J]. Food Res Int, 2023(165): 112540.

[8] FENG J, ZHANG L, XIA X, *et al.* Effect of geographic variation on the proteome of sea cucumber (*Stichopus japonicus*) [J]. Food Res Int, 2020 (136): 109498.

[9] ZHAO Z, LI S, PAN Y, *et al.* Proteomics reveals gender differences in physiological characteristics of the gonads and tube feet of the sea cucumber, *Apostichopus japonicus*[J]. J Proteomics, 2022(251): 104396.

[10] JATTUJAN P, CHALORAK P, SIANGCHAM T, *et al.* Holothuria scabra extracts possess anti-oxidant activity and promote stress resistance and lifespan extension in *Caenorhabditis elegans*[J]. Exp Gerontol, 2018(110): 158-171.

[11] LIN L, YANG K, ZHENG L, *et al.* Anti-aging effect of sea cucumber (*Cucumaria frondosa*) hydrolysate on fruit flies and d-galactose-induced aging mice[J]. Journal of Functional Foods, 2018(47): 11-18.

[12] WU Y, YANG J, XU C, *et al.* Sea cucumber (*Acaudina leucoprocta*) peptides extended the lifespan and enhanced antioxidant capacity via DAF-16/DAF-2/SOD-3/OLD-1/PEPT-1 in *Caenorhabditis elegans*[J]. Front Nutr, 2022(9): 1065145.

[13] WANG X, ZHANG Z, ZHANG S, *et al.* Antiaging compounds from marine organisms[J]. Food Res Int, 2021(143): 110313.

[14] JATTUJAN P, SRISIRIRUNG S, WATCHARAPORN W, *et al.* 2-Butoxytetrahydrofuran and palmitic acid from *Holothuria scabra* enhance *C. elegans* lifespan and healthspan via DAF-16/FOXO and SKN-1/NRF2 signaling pathways[J]. Pharmaceuticals (Basel), 2022(15): 1374.

[15] ASHBURNER M, BALL CA, BLAKE JA, *et al.* Gene ontology: Tool for the unification of biology. The Gene Ontology Consortium[J]. Nature genetics, 2000(25): 25-29.

[16] MI R, SUN Y, LI J, *et al.* Immune-related proteins detected through iTRAQ-based proteomics analysis of intestines from *Apostichopus japonicus* in response to tussah immunoreactive substances[J]. Fish Shellfish Immunol, 2018(74): 436-443.

[17] KANEHISA M, SATO Y, Kawashima M, *et al.* KEGG as a reference resource for gene and protein annotation[J]. Nucleic acids research, 2016(44): D457-462.

[18] CHEN M, ZHU A, STOREY KB. Comparative phosphoproteomic analysis of intestinal phosphorylated proteins in active versus aestivating sea cucumbers[J]. J Proteomics, 2016(135): 141 – 150.
[19] KITISIN T, SUPHAMUNGMEE W, MEEMON K. Saponin-rich extracts from *Holothuria leucospilota* mediate lifespan extension and stress resistance in *Caenorhabditis elegans* via daf-16[J]. J Food Biochem, 2019(43): 3075.
[20] PANGESTUTI R, ARIFIN Z. Medicinal and health benefit effects of functional sea cucumbers[J]. Journal of traditional and complementary medicine, 2018(8): 341 – 351.
[21] BAHRAMI Y, ZHANG W, CHRISTOPHER MMF. Distribution of saponins in the sea cucumber *Holothuria lessoni*; the body wall versus the viscera, and their biological activities[J]. Mar Drugs, 2018(16): 423.
[22] MCCULLY KS. Review: Chemical pathology of homocysteine VI. Aging, cellular senescence, and mitochondrial dysfunction[J]. Annals of clinical and laboratory science, 2018(48): 677 – 687.
[23] WANG CH, WU SB, WU YT, *et al.* Oxidative stress response elicited by mitochondrial dysfunction: Implication in the pathophysiology of aging[J]. Experimental biology and medicine (Maywood, N. J.), 2013(238): 450 – 460.
[24] AMAKYE WK, HOU C, XIE L, *et al.* Bioactive anti-aging agents and the identification of new anti-oxidant soybean peptides[J]. Food Bioscience, 2021(42): 101194.
[25] LAFARGA T, HAYES M. Bioactive peptides from meat muscle and by-products: Generation, functionality and application as functional ingredients[J]. Meat science, 2014(98): 227 – 239.
[26] LU M, MISHRA A, BOSCHETTI C, *et al.* Sea cucumber-derived peptides alleviate oxidative stress in neuroblastoma cells and improve survival in *C. elegans* exposed to neurotoxic paraquat[J]. Oxidative medicine and cellular longevity, 2021(2021): 8842926.
[27] LU Z, SUN N, DONG L, *et al.* Production of bioactive peptides from Sea cucumber and its potential health benefits: A comprehensive review[J]. Journal of agricultural and food chemistry, 2022(70): 7607 – 7625.
[28] LIN X, YAO M, LU JH, *et al.* Identification of novel oligopeptides from the simulated digestion of sea cucumber (*Stichopus japonicus*) to alleviate Aβ aggregation progression[J]. Journal of Functional Foods, 2019(60): 103412.
[29] WANG T, ZHENG L, ZHAO T, *et al.* Anti-diabetic effects of sea cucumber (*Holothuria nobilis*) hydrolysates in streptozotocin and high-fat-diet induced diabetic rats via activating the PI3K/Akt pathway[J]. Journal of Functional Foods, 2020(75): 104224.
[30] ZHANG S, LI F, ZHOU T, *et al.* *Caenorhabditis elegans* as a useful model for studying aging mutations[J]. Frontiers in endocrinology, 2020(11): 554994.
[31] TISSENBAUM HA. Using *C. elegans* for aging research[J]. Invertebrate reproduction & development, 2015(59): 59 – 63.
[32] ZHANG H, PAN N, XIONG S, *et al.* Inhibition of polyglutamine-mediated proteotoxicity by *Astragalus membranaceus* polysaccharide through the DAF-16/FOXO transcription factor in *Caenorhabditis elegans*[J]. The Biochemical journal, 2012(441): 417 – 424.
[33] FATOKUN AA, STONE TW, SMITH RA. Oxidative stress in neurodegeneration and available means of protection[J]. Frontiers in bioscience: a journal and virtual library, 2008, (13): 3288 – 3311.
[34] GUO K, SU L, WANG Y, *et al.* Antioxidant and anti-aging effects of a sea cucumber protein hydrolyzate and bioinformatic characterization of its composing peptides[J]. Food Funct, 2020(11): 5004 – 5016.
[35] PÉREZ-VEGA JA, OLIVERA-CASTILLO L, GÓMEZ-RUIZ JÁ, *et al.* Release of multifunctional peptides by gastrointestinal digestion of sea cucumber (*Isostichopus badionotus*)[J]. Journal of Functional Foods, 2013(5): 869 – 877.
[36] LIN L, ZHU Q, ZHENG L, *et al.* Preparation of sea cucumber (*Stichopus variegates*) peptide fraction with desired organoleptic property and its anti-aging activity in fruit flies and D-galactose-induced aging mice[J]. Journal of Functional Foods, 2020(69): 103954.
[37] LU Z, XU X, LI D, *et al.* Sea cucumber peptides attenuated the scopolamine-induced memory impairment in mice and rats and the underlying mechanism[J]. Journal of agricultural and food chemistry, 2022(70): 157 – 170.
[38] JIN HX, XU HP, LI Y, *et al.* Preparation and evaluation of peptides with potential antioxidant activity by microwave assisted enzymatic hydrolysis of collagen from sea cucumber acaudina molpadioides obtained from Zhejiang Province in China[J]. Mar Drugs, 2019(17): 169.

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fermented chestnut leaf feed, which was rich in nutritional value, and not only provided palatability, but also improved the protein use efficiency of coarse feed and the use efficiency of the feed. After feeding animals, the use efficiency of the fermented chestnut leaf feed increased by a rate equal to greater than 2.11% and the economic profit increased by 22.51%. The animals showed good growth indexes and obvious weight gain, and the economic benefit was greatly improved. The yellow compressed fermented chestnut leaf feed is suitable for wide application and popularization.

References

[1] HAN YS, XU LY, ZHOU J. Present situation and trend of chestnut industry and market development in China[J]. China Fruits, 2021(4): 83 – 88. (in Chinese).
[2] MEYER JB, TRAPIELLO E, SENN-IRLET B, *et al.* Phylogenetic and phenotpic characterisation of *Sirococcus castaneae* comb. Nov. (synonym Diplodina castaneae), a fungal endophyte of European chestnut[J]. Fungal biology, 2017, 121(8): 625 – 637.
[3] LI ZP, WANG TY. Investigation on related factors of chestnut yellow crinkle disease in Huairou District and its comprehensive prevention and control demonstration[J]. China Plant Protection, 2020, 40(10): 65 – 70. (in Chinese).
[4] HE LL, WANG X, LU HN. Analysis of the volatile constituents from the leaves of *Castanea mollissima* BI.[J]. Lishizhen Medicine and Materia Medica Research, 2008, 19(1): 86 – 89. (in Chinese).
[5] QIAN Z, XU HX. High-yield cultivation techniques of chestnut[J]. Modern Agricultural Science and Technology, 2007(17): 33 – 34. (in Chinese).
[6] WANG L. Occurrence characteristics and control countermeasures of chestnut branch diseases in Anhui Province[J]. Flowers, 2017(16): 217 – 218. (in Chinese).
[7] WANG DS, YIN DZ, HE B. Cultivation and management techniques of chestnut orchards in Dabie Mountain area of western Anhui Province[J]. Anhui Agricultural Science Bulletin, 2009(15): 223 – 224. (in Chinese).
[8] DE VASCONCELOS MC, BENNETT RN, ROSA EA, *et al.* Composition of European chestnut (*Castanea sativa* Mill.) and association with health effects: Fresh and processed products[J]. Journal of the Science of Food and Agriculture, 2010, 90(10): 1578 – 1589.

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