Food & Medicine Homology

ISSN 3006-6867 (print); 3006-4252 (online)
https://doi.org/10.26599/FMH.2026.9420086

Research Article

Fermented *Porphyra haitanensis* polysaccharides inhibit the degranulation of mast cell and passive cutaneous anaphylaxis

Zhen Cheng[1], Shao-Han Wang[1], Ling-Ying Li[1], Yu-Chen Zhou[1], Cong-Yi Zhang[1], Hong Liu[1], Tzu-Ming Pan[2], Guang-Ming Liu[1,3], Qing-Mei Liu[1] (✉)

[1] *College of Ocean Food and Biological Engineering, Xiamen Key Laboratory of Marine Functional Food, Fujian Provincial Engineering Technology Research Center of Marine Functional Food, Jimei University, Xiamen 361021, China*
[2] *Department of Biochemical Science and Technology, College of Life Science, Taiwan University, Taibei 10617, Taiwan, China*
[3] *Xiamen Ocean Vocational college, Xiamen 361102, China*

Received: 8 August 2024 / **Revised:** 29 August 2024 / **Accepted:** 29 August 2024

Abstract: Polysaccharides derived from *Porphyra haitanensis* exhibit a range of biological activities. However, they are characterized by high molecular weight and low utilization rates. In the present investigation, the degranulation inhibitory activity of *Porphyra haitanensis* polysaccharides fermented with four distinct *Lactobacillus* strains were evaluated. The fermentation process culminated in the identification of *Lactiplantibacillus plantarum* (CGMCC 11217) as the optimal strain. The optimized fermentation parameters were determined as follows: a solid-to-liquid ratio of 1:40, an inoculum concentration of 1%, and a fermentation duration of 12 hours. Subsequently, the physicochemical properties of the polysaccharides were analyzed. Results indicated a significant reduction in the particle size of the polysaccharide post-fermentation, with an average molecular particle size of 2,619.67 ± 242.37 nm. The rat basophil leukemia (RBL)-2H3 cell model was employed to assess the inhibitory capacity of fermented *Porphyra haitanensis* polysaccharide (FPHSP) on mast cell degranulation. Furthermore, the *in vivo* efficacy of FPHSP was preliminarily evaluated using the passive cutaneous anaphylaxis (PCA) model. Due to the similar properties and inhibitory degranulation activity of purified FPHSP-2 and FPHSP-3, the two components were mixed to create FPHSP-2/3. FPHSP-2/3 was found to diminish the release of *β*-hexosaminidase by inhibiting Ca^{2+} influx in Rat basophil leukemia-2H3 cell (RBL-2H3 cells). Additionally, FPHSP-2/3 mitigated the symptoms of mast cell-mediated passive cutaneous anaphylaxis, implying that FPHSP-2/3 may possess enhanced anti-allergic properties *in vivo*. The present study optimized the fermentation of *Porphyra haitanensis* polysaccharides by *Lactiplantibacillus plantarum*, demonstrating a significant reduction in particle size and enhanced anti-allergic activity *in vitro* and *in vivo*, suggesting potential for improved utilization of these polysaccharides.

Keywords: fermentation; *Lactiplantibacillus plantarum*; mast cell stabilizing; polysaccharides; *Porphyra haitanensis*

Citation: Cheng Z., Wang S. H., Li L. Y., et al. Fermented *Porphyra haitanensis* polysaccharides inhibit the degranulation of mast cell and passive cutaneous anaphylaxis. *Food & Medicine Homology*, 2026, 3: 9420086. https://doi.org/10.26599/FMH.2026.9420086

1 Introduction

The term "blue food" covers a diverse range of plants, animals, and algae that inhabit both freshwater and marine ecosystems. These organisms have the ability to synthesize a wide variety of chemical compounds with potential bioactivity. The diversity and plasticity of blue food reflect its significant potential for future applications in the food industry[1]. The large species of algae are pervasive in aquatic ecosystems, capacity to endure harsh conditions, and adaptability make it ideal subjects for research in areas such as blue food production and functional food ingredients[2]. Seaweed has developed unique metabolic pathways to adapt to the extreme marine environment, characterized by high salinity, high pressure, and low temperatures. This renders it a rich source of metabolites that are not found in terrestrial organisms, particularly polysaccharides. These products possess distinctive biological activities, including antioxidant, antibacterial, and antiviral properties[3]. These properties make it extensively employed in the food, pharmaceutical, and daily chemical industries[4]. However, the preservation effects and biological activity of seaweed polysaccharides are not always satisfactory. Consequently, researchers are proactively seeking effective methods to enhance the biological activity of marine polysaccharides. A substantial body of evidence indicates that the biological activity of marine polysaccharides is associated with their intricate chemical structure[5]. According to extant research, modifying the molecular structure of marine polysaccharides through diverse extraction techniques and molecular modification

Address correspondence to Qing-Mei Liu, liuqingmei@jmu.edu.cn

strategies can impart novel biological properties upon seaweed polysaccharides or augment their intrinsic biological activity[6,7].

Microbial fermentation technology represents a well-established biological modification method. The process of fermentation is defined as a biotransformation process that involves a multitude of reactions, including hydrolysis, oxidation, reduction, esterification, and isomerization. These reactions are catalyzed by various enzymes, such as glycosidases, cellulases, lipases, and proteases, which are produced by microorganisms[8]. The fermentation process may cause the structure of the substrate to be modified[9]. The fermentation method offers several advantages over conventional chemical modification techniques, including the ability to operate under milder reaction conditions, higher specificity, and reduced environmental impact[10]. The term 'probiotic' is used to describe preparations containing defined microorganisms or products that contain viable microorganisms, primarily lactobacilli and bifidobacteria[11]. Furthermore, an increasing number of studies are investigating the potential of using probiotics to ferment plants to obtain bioactive[12]. The application of probiotic fermentation for the biomodification of polysaccharides represents a promising area of research. Microbial fermentation has been shown to enhance the anti-inflammatory activity of Chinese yam polysaccharides[13]. Besides, studies have shown that fermented lentinan exhibits greater protective effects on immunosuppressed mice[14]. Fermented polysaccharides derived from *Polygonatum odoratum* using *Bacillus subtilis* have been identified as a potential source of safe, natural immunostimulants, which enhance the immune capacity of mice through various mechanisms[15]. More than 30 kinds of lactic acid bacteria were screened in the laboratory, and four kinds of *Lactobacillus* bacteria (*Lacticaseibacillus paracasei* (GDMCC 15558), *Lactiplantibacillus plantarum* (CGMCC 11212, CGMCC 11217), *Lactobacillus acidophilus* (BNCC 336636)) could significantly reduce the molecular weight and viscosity of polysaccharide from red algae (*Eucheuma spinosum*, *Gracilaria lemaneiformis*) and improve biological activity[16,17].

The polysaccharides present in seaweed have been demonstrated to exhibit diverse beneficial activities, including anticoagulant, anti-inflammatory, antioxidant, anticarcinogenic, and antiviral activities[18]. Their potential in various biological, biomedical, and functional food applications has been extensively studied[19]. For instance, sulfated oligosaccharides derived from *Gracilaria lemaneiformis* have been found to protect against food allergic responses in mice by promoting immunosuppression[20]. Fucoidan has demonstrated significant anti-tumor and anticoagulant activities[21]. *Eucheuma spinosum* sulfate polysaccharides have also shown anti-food allergy effects[16]. Research on polysaccharides from *Porphyra haitanensis* has revealed multiple biological activities, including antioxidant, lipid-lowering, anticoagulant, and anti-tumor properties[22]. However, the substantial molecular weight of the crude polysaccharides derived from *Porphyra haitanensis* may restrict their biological efficacy[23]. With high yields and minimal costs, there is a possibility for probiotic fermentation to augment the bioactivity of the nutritional components of *Porphyra haitanensis* and expand its utility as a marine functional food.

In this study, we aim to optimize the fermentation conditions for the probiotic fermentation of *Porphyra haitanensis* polysaccharides, analyze the physicochemical properties and functional groups of the fermented polysaccharides, and ultimately verify their mast cell stabilizing activity through *in vitro* cell models and *in vivo* mouse studies.

2 Materials and methods

2.1 Materials and reagents

Porphyra haitanensis collected from Dadeng Island was procured from a local market in Xiamen City, Fujian Province, China. Eagle's Minimum Essential Medium (MEM) was obtained from Hyclone (Logan, UT, USA), while fetal bovine serum (FBS) was sourced from Gemini (Woodland, CA, USA). Anti-dinitrophenyl (DNP)-IgE was purchased from Sigma-Aldrich (St. Louis, MO, USA), and DNP-BSA was acquired from Biosearch (Petaluma, CA, USA). Fluo 3-AM and MRS medium were obtained from Huankai Microbial (Guangdong, Guangzhou, China). Additionally, rat basophil leukemia (RBL)-2H3 cells were provided by Shanghai EK-Bioscience Biotechnology Co., Ltd. (Shanghai, China).

2.2 Bacterial lineages

Lacticaseibacillus paracasei (GDMCC 15558), *Lactiplantibacillus plantarum* (CGMCC 11212, CGMCC 11217), *Lactobacillus acidophilus* (BNCC 336636) were purchased from Xiamen SciplusBiotech. Co., Ltd. (Xiamen, Fujian, China).

2.3 Fermentation preparation of polysaccharides

2.3.1 Strain screening and the condition optimization for fermentation

Lacticaseibacillus paracasei (GDMCC 15558), *Lactiplantibacillus plantarum* (CGMCC 11212, CGMCC 11217), *Lactobacillus acidophilus* (BNCC 336636), four different fermentation strains of the genus Lactobacillus that were used to ferment *Porphyra haitanensis* under the same conditions respectively. Crude polysaccharides were obtained after 4-fold ethanol (95%) precipitation and freeze-dry, and four crude polysaccharides were tested for their ability to inhibit the degranulation of RBL-2H3 cells. Conditions of fermentation significantly influenced the yield of polysaccharides, therefore this study investigated the optimization of fermentation conditions for probiotics. The effects of fermentation time (3 h, 6 h, 12 h, 24 h, 36 h), solid-liquid ratio (1:20, 1:30, 1:40, 1:50, 1:60, 1:70), and inoculation amount (0, 0.1%, 0.2%, 0.5%, 1%, 2%, 4%, 6%, 8%) on the yield of FPHSP were investigated by single factor experiment. An orthogonal $L_9(3)^4$ test design in the extraction mode was used for optimization the extraction conditions. Nine extractions were carried out at solid-liquid ratios of 1:30, 1:40 and 1:50, inoculation amount of 0.5%, 1% and 1.5%, and fermentation time 6 h, 12 h and 18 h.

2.3.2 Extraction and purification of polysaccharides

Porphyra haitanensis was thoroughly cleaned and dried. *Porphyra haitanensis* was fermented according to the optimal fermentation conditions. Following fermentation, a supernatant four volumes of 95% ethanol were added to the supernatant, which was then stored at 4 °C overnight. The resulting precipitate was dissolved in ultrapure water and freeze-dried to produce FPHSP[24]. Based on previously reported techniques, different elution fractions were collected through column chromatography[25]. FPHSP-1 was one component of the ultrapure water elution fractions. The high-salt elution fractions were designated as FPHSP-2, and FPHSP-3. Elution curves of polysaccharides prepared were generated.

2.4 Characterization analysis of polysaccharides

2.4.1 Physicochemical properties analysis

The total sugar content was measured by the anthrone-sulfuric acid method[26]. A 200 μL sample (1 mg/mL) was mixed with 800 μL anthranone-sulfuric acid color developing solution on ice, bathed in water at 100 °C for 10 min, and then the absorbance at 630 nm was determined. The standard curve was drawn with glucose as the standard for calculation. While reducing sugars were quantified using the 3,5-dinitrosalicylic acid colorimetric method[27]. A 600 μL sample (1 mg/mL) was mixed with 900 μL color developing solution, bathed in water at 100 °C for 15 min, and then the absorbance at 520 nm was measured. Using galactose as the standard, the standard curve was drawn for calculation. The sulfate content was assessed using barium chloride turbidimetry[28]. The sample was prepared into 5 mg/mL solution. Taking K_2SO_4 as the standard, the sulfate content was calculated according to the difference between the absorbance value of 360 nm when 5% BaCl-5% gelatin was added and the absorbance value of 360 nm when only 5% gelatin was added.

2.4.2 Particle size analysis

Purified sample solutions of FPHSP, FPHSP-1, FPHSP-2, and FPHSP-3 were prepared at varying concentrations (2.5 mg/mL, 5 mg/mL, 10 mg/mL). The average particle size of these samples was measured using the Zetasizer Nano ZS90 (Malvern Panalytical Technologies, England, UK) at 25 °C.

2.4.3 FT-IR and UV spectra analysis

Fourier-transform infrared spectroscopy (FT-IR) was conducted using an FT-IR spectrometer (Bruker Co., Karlsruhe, BW, Germany). Samples were mixed with KBr at a ratio of 1:100 (*w*/*w*), ground, and pelletized to achieve a particle size of less than 5 mm in diameter, resulting in thin and transparent pellets. These pellets were then mounted on the spectrophotometer for infrared measurements in the range of 4,000-400 cm^{-1}. Additionally, the maximum UV absorption wavelength of the samples was determined at varying concentrations of NaOH solution (0, 0.1, 0.2, 0.3, 0.4, and 0.5 mol/L).

2.5 Inhibitory activity of polysaccharides on mast cell degranulation

2.5.1 Cell culture and viability analysis

The RBL-2H3 cell line was cultured in complete MEM supplemented with 10% FBS and incubated in a humidified atmosphere at 37 °C with 5% CO_2[29]. The effects of different sample concentrations (FPHSP-CGMCC 11217 of 10, 50, 100, 200, 400, 800 and 1,000 μg/mL, FPHSP, FPHSP-1, FPHSP-2, FPHSP-3 of 200, 400 and 800 μg/mL) on the viability of RBL-2H3 cells was assessed using the Cell Counting Kit-8 (CCK-8) assay[30].

2.5.2 Detection of β-hexosaminidase release

RBL-2H3 cells were sensitized with 0.4 μg/mL of anti-DNP-IgE and subsequently challenged with 1 μg/mL of DNP-BSA[31]. The levels of β-hexosaminidase in both the cell supernatant and lysate were measured.

2.5.3 Detection of intracellular calcium concentration

Intracellular calcium levels were measured using the calcium fluorescent probe Fluo3-AM to assess calcium influx[32]. In the RBL-3H3 cell, Fluo-3 formed by the cleavage of calcium ion fluorescence probe Fluo-3AM by lactase can bind to Ca^{2+} and emit strong fluorescence. The change of intracellular Ca^{2+} concentration was determined by observing the intensity of fluorescence with an inverted fluorescence microscope.

2.6 Passive cutaneous anaphylaxis model

Female BALB/c mice (6–8 weeks old) were obtained from the Shanghai Laboratory Animal Center of the Chinese Academy of Sciences (Shanghai, China). The mice were housed in a specific pathogen-free (SPF) environment with stable temperature (22 ± 1 °C) and humidity (55 ± 10%) for one week prior to experimentation. All procedures were conducted in strict accordance with the NIH Guidelines for the care and use of laboratory animals (NIH Publication No. 85–23 Revised 1985). The study protocol was approved by the Institutional Animal Care and Use Committee of the Animal Laboratory Center of Jimei University (Xiamen, China, No. SCXK 2016–0006).

Mice (6–8 weeks old, weighing 18–22 g) were randomly divided into five groups, with researchers blinded to the group assignments. A total of 500 ng of anti-DNP-IgE was injected intradermally into one ear of each mouse. The following day, polysaccharides (20 mg/mL, 200 μL) were administered via intragastric injection three times at 4-hour intervals. One hour after the last administration, a tail vein injection of 200 μL DNP-BSA (5 mg/mL in Evans blue solution containing 0.1 mg/mL DNP-BSA) was performed. One hour after the DNP-BSA injection, after the ears of the dizzy mice were photographed respectively, and the mouse eyeball blood were collected and euthanized. Serum was absorbed after eyeball blood centrifugation (3,500 min, 15 min). All sera were stored at −80 °C. The mouse ears with blue spots were removed, cut into pieces and placed in 2 mL centrifuge tubes. The ear fragments were taken and incubated at 37 °C in KOH for 12 h. Acetone and phosphoric acid solution were mixed at 13:5 and added to the above system to extract dye. The supernatant was centrifugally absorbed. The absorbance value of the supernatant at 620 nm was used to determine the amount of dye in mouse ear. The activity of the polysaccharides was preliminarily evaluated using the passive cutaneous anaphylaxis model based on the swelling of the mouse auricle and the formation of blue spots *in vivo*.

2.7 Statistical analysis

All data are presented as means ± standard deviation. Differences between two groups were analyzed using Student's t-test, while comparisons among multiple groups ($n \geq 3$) were performed using one-way ANOVA followed by Tukey's and Dunnett's multiple comparisons test. In Table 2, we compared the mean of each column with the mean of every other column, and selected Tukey's multiple comparisons test. In other graphs, only the mean of each column and the mean of control column was compared, so Dunnett's multiple comparisons test is selected. Statistical analyses were conducted using GraphPad Prism software, with $P < 0.05$ considered statistically significant.

3 Results

3.1 Strain screening and condition optimization for fermentation

Lacticaseibacillus paracasei (GDMCC 15558), *Lactiplantibacillus*

plantarum (CGMCC 11212, CGMCC 11217), *Lactobacillus acidophilus* (BNCC 336636), four different fermentation strains of the genus Lactobacillus were used to ferment *Porphyra haitanensis* under the same conditions (solid-liquid ratio 1:70, inoculation amount 5%, fermentation time 24 h) individually. Crude polysaccharides were obtained after 4-fold ethanol (95%) precipitation and freeze-dried, and four crude polysaccharides were tested for their ability to inhibit degranulation of RBL-2H3 cells. As shown in Fig. 1A, the release rate of β-hexosaminidase from RBL-2H3 cells in the negative control group (NC group) was 26.96%, while the release rate of β-hexosaminidase from the positive group (PC group） which was stimulated with DNP-BSA, reached 63.86%. There was significant difference between the two groups ($P < 0.000,1$). Compared with the PC group, there was no significant decrease in the release rate of β-hexosaminidase from RBL-2H3 cells after the intervention of crude polysaccharides fermented by *Lacticaseibacillus paracasei* (GDMCC 15558), *Lactobacillus acidophilus* (BNCC 336636). After the intervention of crude polysaccharide from *Lactiplantibacillus plantarum* (CGMCC 11212, CGMCC 11217), the release rate of β-hexosaminidase from RBL-2H3 cells was significantly decreased ($P < 0.01$). Meanwhile, the ability of the two crude polysaccharides to inhibit degranulation of RBL-2H3 cells was not concentration-dependent at concentrations of 100-200 μg/mL. The polysaccharide yield (21.28%) of *Lactiplantibacillus plantarum* (CGMCC 11212) fermentation was lower than that of the CGMCC 11217 fermentation (27.26%). Because of the poor growth of this strain in the fermentation process, the *Lactiplantibacillus plantarum* (CGMCC 11217) was selected to continue the follow-up test.

In order to verify that fermented crude polysaccharide can inhibit degranulation of RBL-2H3 cells, the effects of crude polysaccharides at different concentrations on the viability of RBL-2H3 cells were analyzed. In Fig. 1B, at 10 ~ 1000 μg/mL, crude polysaccharide samples prepared after fermentation with *Lactiplantibacillus plantarum* (CGMCC 11217) had no significant effect on the viability of RBL-2H3, and the cell activity still remained above 90%. FPHSP can inhibit the degranulation of RBL-2H3 cells.

Therefore, the process conditions of FPHSP were further optimized using one-way and orthogonal experiment. The results of single-factor experiments were shown in Figs. 1C-1E. Keeping other conditions unchanged, with an increase of solid-liquid ratio, the yield of FPHSP initially increased, then tended to be stable, and then decreased. Further analysis found that there was no significant difference in the yield of polysaccharide when the ratio of solid-liquid was 1:40, 1:50 and 1:60, so 1:40 was selected as the subsequent test condition. The yield curve of inoculation amount and fermentation time was similar to the trend of solid-liquid ratio. The peak value was reached when the inoculation amount was 0.5%, and then the polysaccharide yield began to decline, so the inoculation amount 0.5% was selected as the subsequent test condition. When fermentation time was 12 h and 24 h, there was no significant difference in the yield of FPHSP, so the fermentation time was 12 h as the subsequent test condition.

The effect of the three factors on the yield of FPHSP was inconsistent. The optimal level combination was A2B2C2 (solid-liquid ratio of 1:40, inoculation amount of 1%, fermentation time of 12 h). In Table 1, the optimal horizontal combination A2B2C2 was not included in the 9 groups of experiment in the orthogonal test. Therefore, three verification experiments were conducted under the conditions of A2B2C2, and the average yield of FPHSP measured was 33.04% ± 1.42%.

In summary, FPHSP inhibited the release of β-hexosaminidase from RBL-2H3 cells. The optimal conditions for polysaccharide preparation from *Porphyra haitanensis* fermentation were solid liquid ratio 1:40, inoculation amount 1% and fermentation time of 12 h.

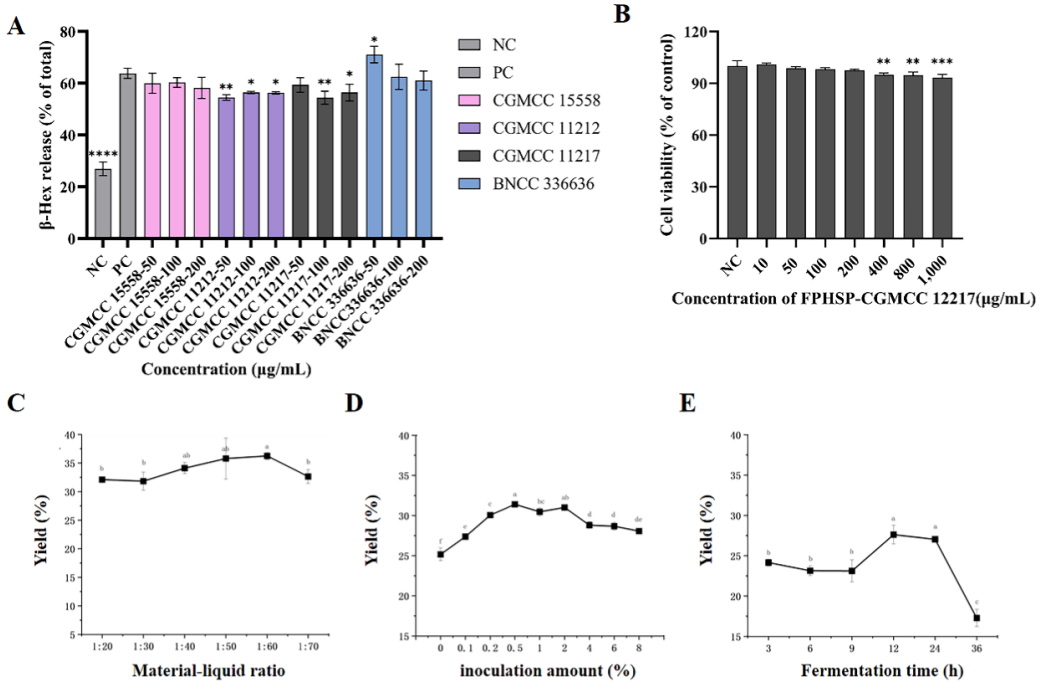

Figure 1 Results of strain screening and condition optimization for fermentation of crude polysaccharide from *Porphyra haitanensis*. (A) Screening results of fermentation of crude polysaccharide from *Porphyra haitanensis,* (B) Effects of FPHSP-CGMCC 11217 on RBL-2H3 viability. The results of single-factor experiments are shown in C-E. Effects of Material-liquid ratio (C), inoculation amount (D), fermentation time (E) on the yield of FPHSP-CGMCC12217. ****$P < 0.000,1$, ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$.

Table 1 Results and analysis of the orthogonal experiment

Trial	A Ratio of material to water (*w*/*v*)	B Inoculation size (%)	C Fermentation time (h)	Yield (%)
1	1:30	0.5	6	26.32
2	1:30	1.0	12	33.40
3	1:30	1.5	18	29.14
4	1:40	0.5	12	33.16
5	1:40	1.0	18	29.59
6	1:40	1.5	6	30.71
7	1:50	0.5	18	30.28
8	1:50	1.0	6	30.43
9	1:50	1.5	12	29.89
K1	88.862,2	89.770,5	87.469,5	
K2	93.459,1	93.422,9	96.450,0	
K3	90.604,4	89.732,3	89.006,2	
k1	29.620,7	29.923,5	29.156,5	
k2	31.153,0	31.141,0	32.150,0	
k3	30.201,5	29.910,8	29.668,7	
R	1.532,3	1.230,2	2.993,5	
Optimal Level		A2B2C2		

3.2 The physicochemical properties of FPHSP and purified components

Porphyra haitanensis polysaccharides fermented under optimal condition was prepared. As the column chromatography showed (Fig. 2A), three more distinct absorption peaks appeared in the polysaccharides of *Porphyra haitanensis* after separation by DEAE-52. FPHSP-1 represented the ultrapure water elution component. FPHSP-2 and FPHSP-3 represented different concentrations of NaCl elution component. Shown in Fig. 2B, the total sugar content of FPHSP-2 (82.83 ± 1.13%) and FPHSP-3 (84.22 ± 0.62%) was similar and significantly higher than that of FPHSP-1 (39.52 ± 1.04%). The sulfate content of crude polysaccharide FPHSP and purified polysaccharide components, FPHSP-1, FPHSP-2 and FPHSP-3 was 13.48 ± 3.53%, 11.97 ± 4.26%, 15.46 ± 1.95% and 24.59 ± 2.89%, respectively. The sulfate content was significantly higher in FPHSP-3 than in FPHSP. Studies have shown that the content of sulfate is closely related to the activity of polysaccharide[33]. FPHSP-3 was supposed to have better activity than FPHSP, FPHSP-1, FPHSP-2.

The fermented polysaccharide and its purified components were tested for the particle sizes (Table 2). The particle sizes of FPHSP-2 and FPHSP-3 increased with the increase in solution concentration. However, there was no significant difference in the particle size between FPHSP-2 and FPHSP-3 solutions in the range of 2.5-5 mg/mL. Zheng had reported that *Porphyra haitanensis* polysaccharide have a particle size about 5.98 μm[34]. This comparison reveals that fermentation significantly reduced particle size of *Porphyra haitanensis* polysaccharide. FPHSP-2 and FPHSP-3 had a similar proper ty in particle sizes.

In Fig. 2C, all four polysaccharides have absorption peaks at 3425 cm^{-1}, which indicates the presence of inter- or intramolecular O-H. FPHSP-1 had a lower absorption peak there. FPHSP, FPHSP-2, and FPHSP-3 had peaks at 2,929 cm^{-1}, which indicates the antisymmetric stretching vibration of C-H, and FPHSP-1 had a less pronounced peak there. C-H bending and stretching vibration peak occurs at 1,465-1,340 cm^{-1}. FPHSP, FPHSP-1, FPHSP-2 and FPHSP-3 all had peaks at 1,071 cm^{-1} indicating strong characteristic absorption peaks of C-O, but the peak of FPHSP-1 is not obvious at this location. The appearance of all these absorption peaks indicated that FPHSP, FPHSP-1, FPHSP-2 and FPHSP-3 were all saccharides. Meanwhile, FPHSP, FPHSP-2 and FPHSP-3 all had obvious absorption peaks at 1641 cm^{-1} for COO-. FPHSP, FPHSP-2 and FPHSP-3 all had characteristic

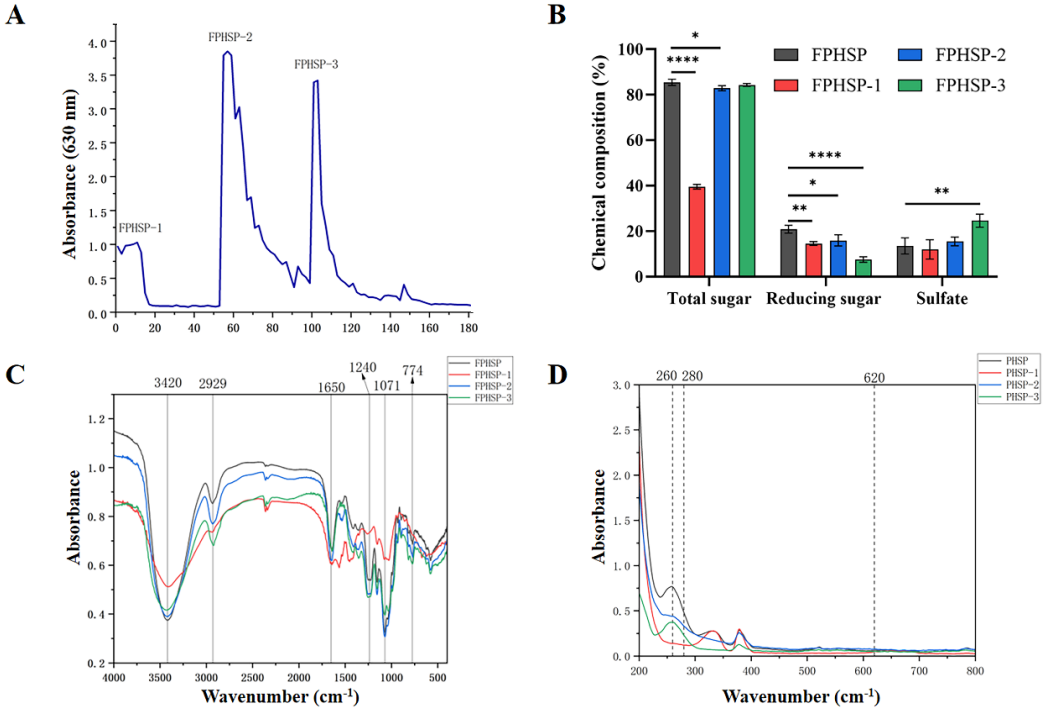

Figure 2 Results of purification curve and chemical composition of polysaccharide from fermented *Porphyra haitanensis*. (A) DEAE-52 column chromatography purification curves of FPHSP. (B) Chemical composition of FPHSP, FPHSP-1, FPHSP-2 and FPHSP-3. (C) Fourier transform infrared spectroscopy of FPHSP, FPHSP-1, FPHSP-2 and FPHSP-3. (D) UV spectra of FPHSP, FPHSP-1, FPHSP-2 and FPHSP-3. ****$P < 0.000,1$, ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$.

Table 2 Effects of fermentation on the particle sizes of FPHSP

Items		FPHSP	FPHSP-1	FPHSP-2	FPHSP-3
Particle size (nm)	10 mg/mL	4,605.33 ± 454.00^{a}	2,040.00 ± 874.69^{c}	2,718.00 ± 294.90b^{c}	3,834.00 ± 57.69^{ab}
	5 mg/mL	2,619.67 ± 242.37^{ab}	3,060.93 ± 257.39^{a}	1,727.67 ± 14.57^{b}	2,270.00 ± 81.28^{ab}
	2.5 mg/mL	1,935.33 ± 165.45^{b}	10,028.67 ± 636.40^{a}	1,532.67 ± 110.80^{b}	1,763.33 ± 11.49^{b}

Note: Values are expressed as means ± SD and three replicated independent determinations.
Values with different letters in the same line indicate statistically significant differences ($P < 0.05$).

absorption peaks for sulfate near 1,240 cm^{-1} and 774 cm^{-1}, while FPHSP-1 does not have obvious absorption peaks at these two places. The comparison shows that the functional groups of FPHSP-2 did not change with those of FPHSP-3.

In Fig. 2D, UV full wavelength scanning spectra were used to determine the constituents of samples. FPHSP, FPHSP-2, and FPHSP-3 peaked at 260 nm, indicating that they contain a small number of nucleic acids. FPHSP-1 had no obvious peak at 260 nm, so the sample basically did not contain nucleic acids. Four groups of samples had no obvious peak at 280 nm, so they basically did not contain protein.

3.3 RBL-2H3 cells stabilization activities of FPHSP

3.3.1 FPHSP decreased degranulation of stimulated mast cells

The activity of purified and unpurified fermented *Porphyra haitanensis* polysaccharides was tested *in vitro*. The effects of FPHSP and its purified components on the viability of RBL-2H3 cells were detected by CCK-8. Additionally, the effects of polysaccharides samples at different concentrations on the viability of RBL- 2H3 cells were analyzed. As shown in Fig. 3A, the four samples had no significant effect on the viability of RBL-2H3 cells. And the cell viability was still above 90%, proving samples had no significant effect on cell viability.

In Fig. 3B, compared with NC group, the release rate of *β*-hexosaminidase from RBL-2H3 cells in the PC group was significantly increased. The efficiency of RBL-2H3 releasing *β*-aminocaproic glycosidase decreased significantly after the intervention of FPHSP-1, FPHSP-2 and FPHSP-3. While FPHSP-2 at 800 μg/mL showed better mast cell inhibitory activity than FPHSP-3 ($P < 0.05$).

3.3.2 FPHSP-2/3 reduced calcium influx in stimulated RBL-2H3 cells

The increase of intracellular Ca^{2+} levels is an important factor for mast cell degranulation and other signal transduction[16]. The Ca^{2+} fluorescent probe Fluo-3 AM penetrates the cell membrane into the cell and was cleaved by lactase to form Fluo-3, which is retained in the cell. In the cell, Fluo-3 binds to Ca^{2+} and emits strong fluorescence. According to this principle, the RBL-2H3 cell model was used to explore the effects of fermented *Porphyra haitanensis* polysaccharide samples on Ca^{2+} influx. The purified components FPHSP-2 and FPHSP-3 had similar properties and degranulation inhibition activities, so the two components were mixed to create FPHSP-2/3 to explore the ability to inhibit Ca^{2+} influx in RBL-2H3 cells. As shown in Fig. 3C, the intracellular Ca^{2+} concentration in RBL-2H3 cells declined significantly by the intervention of FPHSP-2/3. Above conclusions prove that FPHSP-2/3 had good inhibitory activity on mast cells *in vitro*.

3.4 FPHSP-2/3 relieved passive cutaneous anaphylaxis in mice

The antiallergic activity *in vivo* of *Porphyra haitanensis* polysaccharide samples was further tested by passive cutaneous anaphylaxis in mice. There was no obvious blue spot in the auricle of mice in the NC group (Fig. 4A), but that of the PC group was obvious. FPHSP-2/3 intervention can inhibit the ear blue spot symptoms of passive cutaneous anaphylaxis model mice. The quantitative results of Evans blue dye extravasation and histamine level of serum of mice were shown in Figs. 4B-4C, Evens blue dye extravasation and histamine of the FPHSP-2/3 intervention group were significantly lower than in the PC group. The results showed

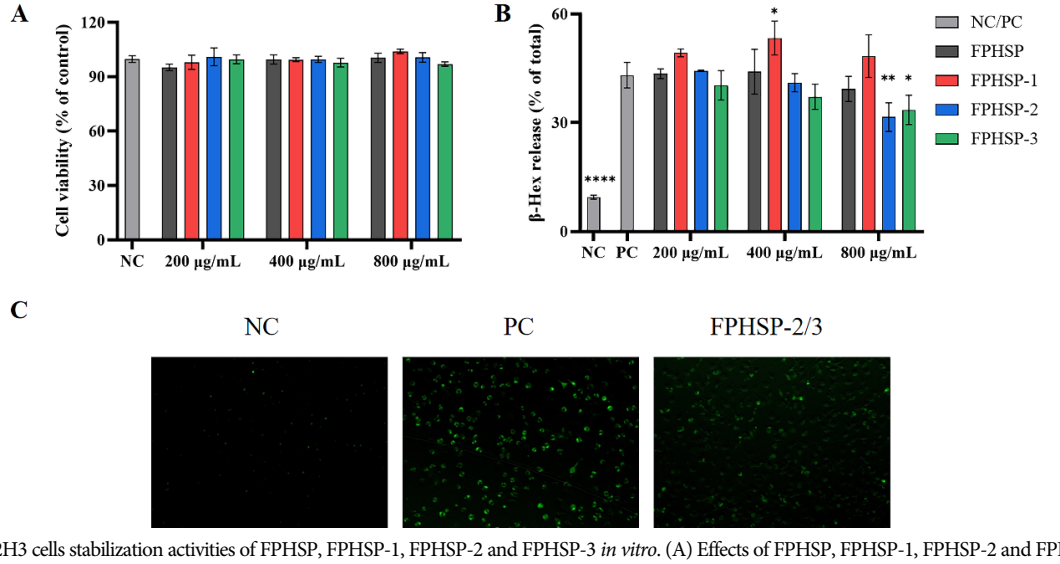

Figure 3 RBL-2H3 cells stabilization activities of FPHSP, FPHSP-1, FPHSP-2 and FPHSP-3 *in vitro*. (A) Effects of FPHSP, FPHSP-1, FPHSP-2 and FPHSP-3 on RBL-2H3 viability. (B) *β*-Hexosaminidase release rate of RBL-2H3. (C) Effects of FPHSP-2/3 on intracellular Ca^{2+} fluorescence intensity. ****$P < 0.000,1$, **$P < 0.01$, *$P < 0.05$.

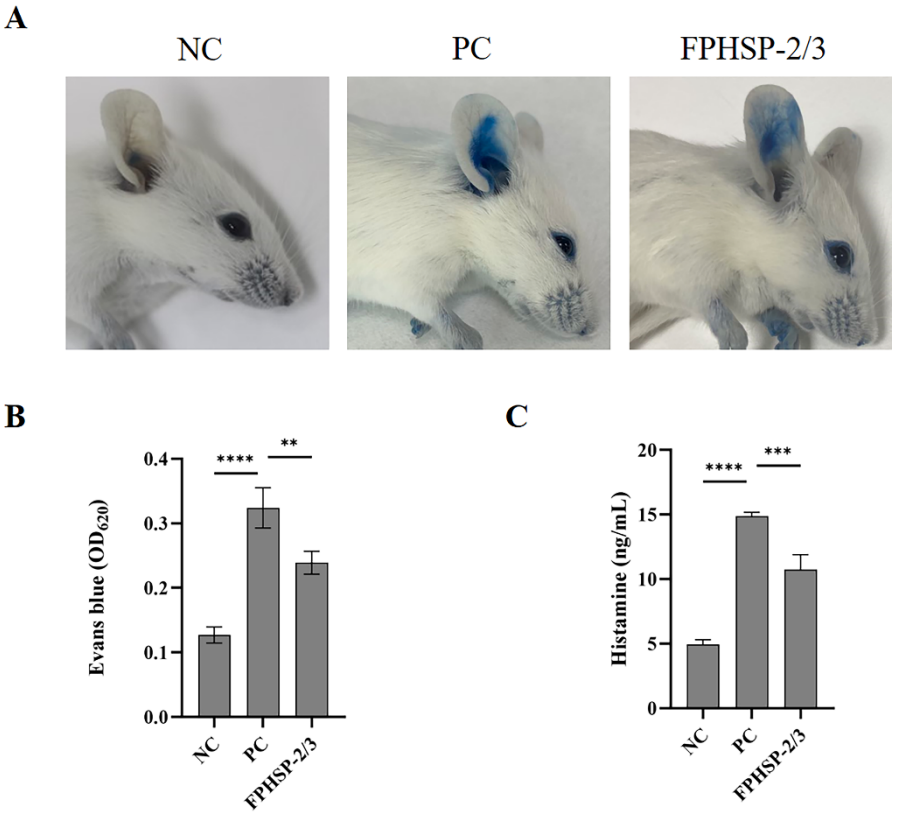

Figure 4 Effects of FPHSP-2/3 on anti-DNP-IgE/DNP-BSA induced passive cutaneous anaphylaxis reaction. (A) The ears of mice with absorbed Evans blue were photographed. (B) Quantitative analysis of Evans blue dye extracts in mouse ears. (C) Histamine level of serum of mice. ****$P < 0.000,1$, ***$P < 0.001$, **$P < 0.01$.

that FPHSP-2/3 could significantly relieve passive cutaneous anaphylaxis and has the ability to stabilize mast cells and inhibit mast cell activation *in vivo*.

4 Discussion

Seaweed, as a crucial component of the "blue food" category, holds significant importance in the realm of functional and specialized food research and development[35]. Studies have highlighted the diverse biological activities of seaweed polysaccharides, including immune regulation, anti-inflammatory effects, and anti-tumor properties[36, 37]. The efficacy of these activities is intricately linked to the polysaccharide preparation methods used, as variations in preparation techniques can impact both the physicochemical characteristics and biological functions of the polysaccharides[38]. In addition to traditional methods such as water extraction and alcohol precipitation, novel approaches like ultrasonic extraction, alkali extraction, and probiotic fermentation offer distinctive avenues for polysaccharide extraction[39]. Probiotic fermentation, in particular, represents a unique biological method that leverages the enzymatic capabilities of probiotics to catalyze the transformation of dietary components during fermentation[40]. This process facilitates a notable increase in the solubility of bioactive compounds, reduction in molecular weight, enhancement of absorption properties, and consequent improvement in biological activity[41]. Furthermore, probiotic fermentation stands out for its mild reaction conditions, specificity, and eco-friendliness, rendering it a more advantageous alternative to chemical modification methods[42]. Research has found that the fermentation of seaweed by probiotics can produce prebiotics and enhance biological activity[43]. Yue *et al*[44] reported that fermented seaweed extracts perform a potent hypolipidemic action. The previous work of our research group also found that the fermentation of red algae by probiotics can reduce the particle size and viscosity of polysaccharides, and enhance anti-allergic activity[16, 17]. Thus, whether the use of different probiotics on *Porphyra haitanensis* could alter the properties and biological functions of polysaccharides is worth looking forward to.

The fermentation of food materials with suitable probiotic strains is an effective way to improve biological activities. By screening the bioactivity of four different FPHSP (*Lacticaseibacillus paracasei* GDMCC 15558, *Lactiplantibacillus plantarum* CGMCC 11212, CGMCC 11217, *Lactobacillus acidophilus* BNCC 336636), we finally selected *Lactobacillus plantarum* (CGMCC 11217) for the fermentation. *Lactiplantibacillus plantarum* (CGMCC 11217) is an important type of Lactobacillus spp. *L. plantarum* is commonly used for probiotic fermentation because of its excellent acid resistance, adhesion, antioxidant and antimicrobial properties. Polysaccharides were extracted from *Porphyra haitanensis* by fermentation of *Lactiplantibacillus plantarum* (CGMCC 11217). The physicochemical properties of polysaccharides extracted after fermentation were analyzed. Meanwhile, the RBL-2H3 cell model and animal model PCA were used to investigate the anti-allergic activity of fermented polysaccharide. It has been previously published that *L. plantarum* fermentation is able to reduce molecular weight, improve water solubility, reduce viscosity and enhance stability by altering glyoxylate content, affecting monosaccharide composition and ratio. Zheng had reported that *Porphyra haitanensis* polysaccharide have a particle size about 5.98 μm[34]. *Porphyra haitanensis* had a particle size of 2,619.67 ± 242.37 nm at 2.5 mg/mL after *Lactiplantibacillus plantarum*

(CGMCC 11217) fermentation. This comparison reveals that fermentation significantly reduced particle size of *Porphyra haitanensis* polysaccharide. Furthermore, antioxidant activity and immunomodulatory activity were enhanced by *L. plantarum* fermentation[45]. Yu *et al*[46] had reported that *L. paracasei* fermentation could increase antioxidant activity remarkably by increasing polysaccharide solubility and reducing their Mws, particle sizes, and apparent viscosity. *L. paracasei* metabolites were reported that it could inhibit a wide range of pathogens and modulate the mucosal immune system authorization of this technology (China authorized patent number: EP 3 366 143 A1, EP 3 470 074 A1). And milk fermentation products of *L. acidophilus* were proved with antioxidant activity[47]. Our results were similar to those of the study above. Compared with previous laboratory studies, fermentation of *Lactiplantibacillus plantarum* (CGMCC 11217), the mast cell stabilization activity (degranulation inhibition rate) of FPHSP significantly improved[48].

Porphyra haitanensis is a red alga with high economic value in the southeastern coastal region of China. Protein, polysaccharides, minerals and vitamins are abundant in *Porphyra haitanensis*. The immunomodulatory effects of *Porphyra haitanensis*, such as antioxidant, hypolipidemic, hypoglycemic, etc., have received widespread attention from scholars in various countries[49]. Previous studies have shown that food allergy could be prevented by *Porphyra haitanensis* polysaccharides regulating Th1/Th2 immune balance. But inhibition of allergic effector cells by the polysaccharides was not significant. Preliminary experiments proved that the inhibition of mast cell degranulation of *Porphyra haitanensis* polysaccharides could be enhanced by probiotic fermentation. Thus, different strains of bacteria fermented *Porphyra haitanensis* polysaccharides were mined in the present studies. RBL-2H3 cells were used as a routine mast cell activation model study due to their FceRI-rich surface. This issue proved that *Porphyra haitanensis* polysaccharides fermented by strain CGMCC 11217 could inhibit the degranulation and Ca^{2+} influx of RBL-2H3 cells. Furthermore, passive cutaneous anaphylaxis in mice was a simple allergic reaction mediated by mast cells and can be used to assess the effects of food components on mast cell activation *in vivo*[50]. The result demonstrated that CGMCC 11217-fermented *Porphyra haitanensis* polysaccharides were able to inhibit the PCA *in vivo*, showing mast cell inhibitory effects and anti-allergic effects.

5 Conclusions

Summarily, screening of degranulation inhibitory activity of *Porphyra haitanensis* polysaccharides fermented by four different Lactobacillus, we found fermentation by strain *Lactiplantibacillus plantarum* (CGMCC 11217) dramatically increased the antiallergic activity of *Porphyra haitanensis* polysaccharide. The high salt elution component of fermented sulfated polysaccharide of *Porphyra haitanensis*, FPHSP-2/3, inhibited the degranulation and Ca^{2+} inflow of RBL-2H3 cells. The symptoms of mast cell-mediated PCA were also significantly relieved by this polysaccharide. *Porphyra haitanensis* polysaccharide fermented by strain CGMCC 11217 can effectively inhibit mast cell's activation. This study is expected to contribute to the development of marine development marine functional foods that can be used to treat environmental allergic diseases and mastocytosis.

Conflicts of interest

The authors declare that there are no conflicts of interest in this work. Guangming Liu is an editor of Food & Medicine Homology, but was not involved in the processing, peer review or decision making of this article.

Acknowledgments

This work was supported by the National Natural Scientific Foundation of China (No. 32472334, 32472449), the Joint Funds of National Natural Scientific Foundation of China (No. U23A20266), and the Science and Technology Program of Xiamen City (No. 2023CXY0307).

References

[1] Crona, B. I., Wassénius, E., Jonell, M., et al. Four ways blue foods can help achieve food system ambitions across nations. *Nature*, **2023**, 616: 104–112. https://doi.org/10.1038/s41586-023-05737-x

[2] Naseem, S., Rizwan, M., Durrani, A. I., et al. Green approaches to blue food (Seaweed) protein processing and advances in purification techniques. *Sustainable Chemistry and Pharmacy*, **2024**, 38. https://doi.org/10.1016/j.scp.2024.101496

[3] Lomartire, S., Goncalves, A. M. M. An Overview of Potential Seaweed-Derived Bioactive Compounds for Pharmaceutical Applications. *Marine Drugs*, **2022**, 20. https://doi.org/10.3390/md20020141

[4] Chi, Y., Jiang, Y., Wang, Z., et al. Preparation, structures, and biological functions of rhamnan sulfate from green seaweed of the genus *Monostroma*: A review. *International Journal of Biological Macromolecules*, **2023**, 249. https://doi.org/10.1016/j.ijbiomac.2023.125964

[5] Zhang, B., Lan, W., Xie, J. Chemical modifications in the structure of marine polysaccharide as serviceable food and assistant: A review. *International Journal of Biological Macromolecules*, **2022**, 223: 1539–1555. https://doi.org/10.1016/j.ijbiomac.2022.11.034

[6] Zhou, T., Li, X. Chemically modified seaweed polysaccharides: Improved functional and biological properties and prospective in food applications. *Comprehensive Reviews in Food Science and Food Safety*, **2024**, 23: e13396. https://doi.org/10.1111/1541-4337.13396

[7] Otero, P., Carpena, M., Garcia-Oliveira, P., et al. Seaweed polysaccharides: Emerging extraction technologies, chemical modifications and bioactive properties. *Critical Reviews in Food Science and Nutrition*, **2023**, 63: 1901–1929. https://doi.org/10.1080/10408398.2021.1969534

[8] Liu, S., Hu, J., Zhong, Y., et al. A review: Effects of microbial fermentation on the structure and bioactivity of polysaccharides in plant-based foods. *Food Chemistry*, **2024**, 440: 137453. https://doi.org/10.1016/j.foodchem.2023.137453

[9] Gao, H., Wen, J., Hu, J., et al. Polysaccharide from fermented *Momordica charantia* L. with *Lactobacillus plantarum* NCU116 ameliorates type 2 diabetes in rats. *Carbohydrate polymers*, **2018**, 201: 624–633. https://doi.org/10.1016/j.carbpol.2018.08.075

[10] Yang, J., Zhang, X., Dai, J., et al. Effect of fermentation modification on the physicochemical characteristics and anti-aging related activities of *Polygonatum kingianum* polysaccharides. *International Journal of Biological Macromolecules*, **2023**, 235: 123661. https://doi.org/10.1016/j.ijbiomac.2023.123661

[11] Lacroix, C., Yildirim, S. Fermentation technologies for the production of probiotics with high viability and functionality. *Current Opinion in Biotechnology*, **2007**, 18: 176–183. https://doi.org/10.1016/j.copbio.2007.02.002

[12] Champagne, C. P., Da Cruz, A. G., Daga, M. Strategies to improve the functionality of probiotics in supplements and foods. *Current Opinion in Food Science*, **2018**, 22: 160–166. https://doi.org/10.1016/j.cofs.2018.04.008

[13] Bai, Y., Zhou, Y., Zhang, R., et al. Gut microbial fermentation promotes the intestinal anti-inflammatory activity of Chinese yam

polysaccharides. *Food Chemistry*, **2023**, 402: 134003. https://doi.org/10.1016/j.foodchem.2022.134003

[14] Liang, J., Zhang, M., Li, X., et al. Structure and immunomodulatory activity of *Lentinus edodes* polysaccharides modified by probiotic fermentation. *Food Science and Human Wellness*, **2024**, 13: 421–433. https://doi.org/10.26599/fshw.2022.9250036

[15] Li, Y., Liu, Y., Liu, M., et al. *Polygonatum odoratum* fermented polysaccharides enhance the immunity of mice by increasing their antioxidant ability and improving the intestinal flora. *Food Bioscience*, **2024**, 58. https://doi.org/10.1016/j.fbio.2024.103650

[16] Zhang, J., Liu, Q., Gu, F., et al. Effects of *Lactobacillus* fermentation on *Eucheuma spinosum* polysaccharides: Characterization and mast cell membrane stabilizing activity. *Carbohydrate polymers*, **2023**, 310: 120742. https://doi.org/10.1016/j.carbpol.2023.120742

[17] Chen, H., Zhou, Y., Liu, Y., et al. Fermented *Gracilaria lemaneiformis* polysaccharides alleviate food allergy by regulating Treg cells and gut microbiota. *International Journal of Biological Macromolecules*, **2024**, 269: 132215. https://doi.org/10.1016/j.ijbiomac.2024.132215

[18] Tanna, B., Mishra, A. Nutraceutical Potential of Seaweed Polysaccharides: Structure, Bioactivity, Safety, and Toxicity. *Comprehensive Reviews in Food Science and Food Safety*, **2019**, 18: 817–831. https://doi.org/10.1111/1541-4337.12441

[19] Venkatesan, J., Anil, S., Kim, S.K., et al. Seaweed Polysaccharide-Based Nanoparticles: Preparation and Applications for Drug Delivery. *Polymers* (*Basel*), **2016**, 8. https://doi.org/10.3390/polym8020030

[20] Liu, Q., Zhang, Y., Shu, Z., et al. Sulfated oligosaccharide of *Gracilaria lemaneiformis* protect against food allergic response in mice by up-regulating immunosuppression. *Carbohydrate polymers*, **2020**, 230: 115567. https://doi.org/10.1016/j.carbpol.2019.115567

[21] Luthuli, S., Wu, S., Cheng, Y., et al. Therapeutic Effects of Fucoidan: A Review on Recent Studies. *Marine Drugs*, **2019**, 17. https://doi.org/10.3390/md17090487

[22] Wu, Y., Huo, Y., Xu, L., et al. Purification, characterization and antioxidant activity of polysaccharides from Porphyra haitanensis. *International Journal of Biological Macromolecules*, **2020**, 165: 2116–2125. https://doi.org/10.1016/j.ijbiomac.2020.10.053

[23] Sun, M., Zhang, Y., Gao, W., et al. Polysaccharides from *Porphyra haitanensis*: A review of their extraction, modification, structures, and bioactivities. *Molecules*, **2024**, 29. https://doi.org/10.3390/molecules29133105

[24] Anwar, M., Mcconnell, M., Bekhit, A. E. New freeze-thaw method for improved extraction of water-soluble non-starch polysaccharide from taro (*Colocasia esculenta*): Optimization and comprehensive characterization of physico-chemical and structural properties. *Food Chemistry*, **2021**, 349: 129210. https://doi.org/10.1016/j.foodchem.2021.129210

[25] Liu, Y., Liu, W., Wang, Y., et al. Inhibitory Effect of Depolymerized Sulfated Galactans from Marine Red Algae on the Growth and Adhesion of Diarrheagenic *Escherichia coli*. *Marine Drugs*, **2019**, 17. https://doi.org/10.3390/md17120694

[26] Morris, D. L. Quantitative determination of carbohydrates with Dreywood's anthrone reagent. *Science*, **1948**, 107: 254–255. https://doi.org/10.1126/science.107.2775.254

[27] Khatri, K., Rathore, M. S., Agrawal, S., et al. Sugar contents and oligosaccharide mass profiling of selected red seaweeds to assess the possible utilization of biomasses for third-generation biofuel production. *Biomass & Bioenergy*, **2019**, 130. https://doi.org/10.1016/j.biombioe.2019.105392

[28] Dodgson, K. S., Price, R. G. A note on the determination of the ester sulphate content of sulphated polysaccharides. *Biochemical Journal*, **1962**, 84: 106–110. https://doi.org/10.1042/bj0840106

[29] Shu, Z., Liu, Q., Xing, C., et al. Viridicatol isolated from deep-sea penicillium griseofulvum alleviates anaphylaxis and repairs the intestinal barrier in mice by suppressing mast cell activation. *Marine Drugs*, **2020**, 18. https://doi.org/10.3390/md18100517

[30] Stockert, J. C., Blázquez-Castro, A., Cañete, M., et al. MTT assay for cell viability: Intracellular localization of the formazan product is in lipid droplets. *Acta Histochemica*, **2012**, 114: 785–796. https://doi.org/10.1016/j.acthis.2012.01.006

[31] Zhang, Y., Liu, Q., Gao, Y., et al. Attenuation of allergic responses following treatment with resveratrol in anaphylactic models and IgE-mediated mast cells. *Food Function*, **2019**, 10: 2030–2039. https://doi.org/10.1039/c9fo00077a

[32] Zhang, Y., Liu, Q., Liu, B., et al. Dihydromyricetin inhibited ovalbumin-induced mice allergic responses by suppressing the activation of mast cells. *Food Function*, **2019**, 10: 7131–7141. https://doi.org/10.1039/c9fo01557d

[33] Huang, L., Shen, M., Morris, G. A., et al. Sulfated polysaccharides: Immunomodulation and signaling mechanisms. *Trends in Food Science & Technology*, **2019**, 92: 1–11. https://doi.org/10.1016/j.tifs.2019.08.008

[34] Zheng, M., Ma, M., Yang, Y., et al. Structural characterization and antioxidant activity of polysaccharides extracted from Porphyra haitanensis by different methods. *International Journal of Biological Macromolecules*, **2023**, 242: 125003. https://doi.org/10.1016/j.ijbiomac.2023.125003

[35] Soares Dias, A. P., Rijo, B., Santos, F., et al. Overview on biofuels production in a seaweed biorefinery. *Science of the Total Environment*, **2023**, 884: 163714. https://doi.org/10.1016/j.scitotenv.2023.163714

[36] Mapelli-Brahm, P., Gómez-Villegas, P., Gonda, M. L., et al. Microalgae, seaweeds and aquatic bacteria, archaea, and yeasts: Sources of carotenoids with potential antioxidant and anti-inflammatory health-promoting actions in the sustainability era. *Marine Drugs*, **2023**, 21. https://doi.org/10.3390/md21060340

[37] Bukhari, N. T. M., Rawi, N. F. M., Hassan, N. a. A., et al. Seaweed polysaccharide nanocomposite films: A review. *International Journal of Biological Macromolecules*, **2023**, 245: 125486. https://doi.org/10.1016/j.ijbiomac.2023.125486

[38] Xu, Y., Wu, Y., Sun, P., et al. Chemically modified polysaccharides: Synthesis, characterization, structure activity relationships of action. *International Journal of Biological Macromolecules*, **2019**, 132: 970–977. https://doi.org/10.1016/j.ijbiomac.2019.03.213

[39] Chen, X., Shen, M., Yu, Q., et al. Recent advance in chemistry modified methods of natural polysaccharides and their applications. *Trends in Food Science & Technology*, **2024**, 144: 104317. https://doi.org/10.1016/j.tifs.2023.104317

[40] Ru, Y., Wang, Z., Li, Y., et al. The influence of probiotic fermentation on the active compounds and bioactivities of walnut flowers. *Journal of Food Biochemistry*, **2022**, 46: e13887. https://doi.org/10.1111/jfbc.13887

[41] Kolodziejczyk, A. A., Zheng, D., Elinav, E. Diet-microbiota interactions and personalized nutrition. *Nature Reviews Microbiology*, **2019**, 17: 742–753. https://doi.org/10.1038/s41579-019-0256-8

[42] Hur, S. J., Lee, S. Y., Kim, Y. C., et al. Effect of fermentation on the antioxidant activity in plant-based foods. *Food Chemistry*, **2014**, 160: 346–356. https://doi.org/10.1016/j.foodchem.2014.03.112

[43] Kim, N. Y., Kim, J. M., Son, J. Y., et al. Synbiotic fermentation of *Undaria pinnatifida* and *Lactobacillus brevis* to produce prebiotics and probiotics. *Applied Biochemistry and Biotechnology*, **2023**, 195: 6321–6333. https://doi.org/10.1007/s12010-023-04415-y

[44] Yue, Q., Wang, Z., Tang, X., et al. Hypolipidemic effects of fermented seaweed extracts by *Saccharomyces cerevisiae and Lactiplantibacillus plantarum*. *Frontiers in Microbiology*, **2021**, 12: 772585. https://doi.org/10.3389/fmicb.2021.772585

[45] Wang, Z., Zhou, X., Sheng, L., et al. Effect of ultrasonic degradation on the structural feature, physicochemical property and bioactivity of plant and microbial polysaccharides: A review. *International Journal of Biological Macromolecules*, **2023**, 236: 123924. https://doi.org/10.1016/j.ijbiomac.2023.123924

[46] Yu, Y., Zhu, Z., Xu, Y., et al. Effects of *Lactobacillus plantarum* FM 17 fermentation on jackfruit polysaccharides: Physicochemical, structural, and bioactive properties. *International Journal of*

Biological Macromolecules, **2024**, 258: 128988. https://doi.org/10.1016/j.ijbiomac.2023.128988

[47] Mathur, H., Beresford, T. P., Cotter, P. D. Health benefits of lactic acid bacteria (LAB) Fermentates. *Nutrients*, **2020**, 12. https://doi.org/10.3390/nu12061679

[48] Liu, Q., Zhou, Y., Gao, Y., et al. Degraded *Porphyra haitanensis* sulfated polysaccharide relieves ovalbumin-induced food allergic response by restoring the balance of T helper cell differentiation. *Food Function*, **2021**, 12: 4707–4719. https://doi.org/10.1039/d1fo00335f

[49] Gong G., Dang T., Fang J., et al. Preparation, structural characterization, and bioactivity of PHPD-IV-4 derived from Porphyra haitanensis. *Food Chemistry*, **2020**, 329: 127042. https://doi.org/10.1016/j.foodchem.2020.127042

[50] Kim, E. A., Han, E. J., Kim, J., et al. Anti-allergic effect of 3,4-dihydroxybenzaldehyde isolated from *Polysiphonia morrowii* in IgE/BSA-Stimulated mast cells and a passive cutaneous anaphylaxis mouse model. *Marine Drugs*, **2022**, 20. https://doi.org/10.3390/md20020133