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Structural properties and immunomodulatory activity of an α -glucan purified from the fruiting body of *Stropharia rugosoannulata*

Zhang Zuofa^{1*†}, Song Tingting^{1†}, Lv Guoying¹, Liu Jie¹ and Jin Qunli¹

Abstract

A novel polysaccharide PSRa-2 was purified from *Stropharia rugosoannulata* fruiting bodies using high pressure homogenization-assisted dual enzyme method, ion exchange, and gel chromatography. The PSRa-2 was characterized via FT-IR, HPAEC, SEM, Congo red test, SEC-MALLS-RI, methylation analysis, and NMR analysis. Structural characterization revealed that PSRa-2 was an α -glucan with a Mw 455.6 kDa. The backbone of PSRa-2 was composed of $\rightarrow 4$ - α -D-Glcp-(1 \rightarrow and $\rightarrow 3$)- α -D-Glcp-(1 \rightarrow and branches of α -D-Glcp-(1 \rightarrow at position O-6 of $\rightarrow 4,6$)- α -D-Glcp-(1 \rightarrow . PSRa-2 induced splenocyte proliferation and protected splenocytes against 5-Fu-induced immunosuppression by restoring the proliferation and secretion of cytokines (TNF- α and IL-2) secretion levels. Thus, PSRa-2 exhibits obviously immunomodulatory activity and represents a potential natural immunomodulator.

Keywords *Stropharia rugosoannulata*, Structural properties, α -D-Glucan, Immunomodulatory activity

Zhang Zuofa and Song Tingting contributed equally to this work.

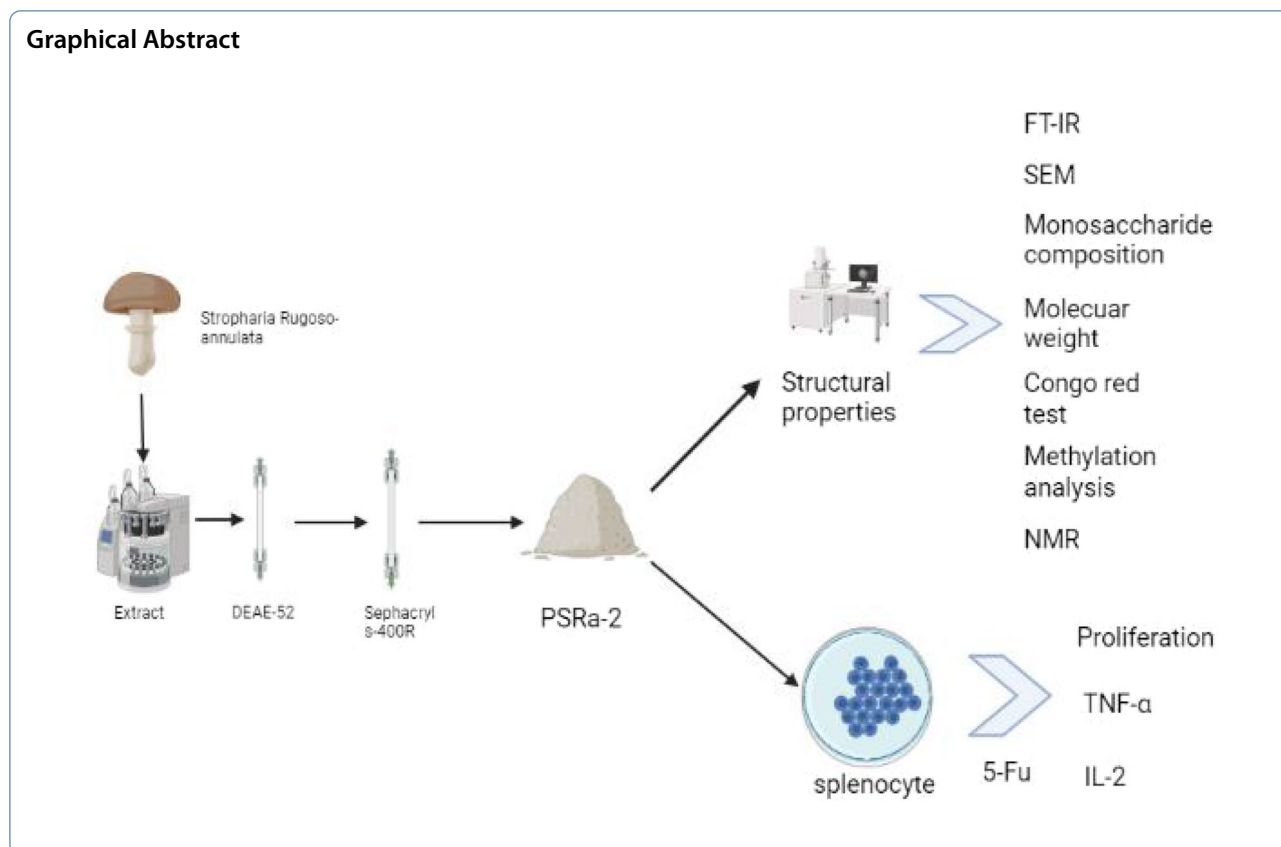
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Introduction

It was well known that the imbalance in immunoregulation leads to a variety of diseases [1]. Therefore, the modulation of immune response has long been considered as a crucial defense against disease [2]. Treatment of immunomodulators is an important strategy to improve immunity. Further evidence suggests that many compounds or extracts isolated from mushrooms can activate host immune responses [3, 4]. They are safe and facilitate adaptation to environmental and biological stress. Currently, the natural polysaccharides are the focus of attention due to their various pharmacological properties [5, 6]. Many studies have reported that the polysaccharides from mushroom can activate lymphocytes, regulate the cytokines levels and stimulate the activation of phagocytes.

Stropharia rugosoannulata, a delicious mushroom, has been widely cultivated in many countries. In recent years, the consumption of *S. Rugosoannulata* has increased dramatically due to its dainty taste and pharmacological activities [7, 8]. *S. rugosoannulata* is rich in polysaccharides, dietary fiber, trace elements,

and vitamins [9]. As the main ingredient, the polysaccharide derived from *S. rugosoannulata* possesses various pharmaceutical properties, including anti-tumor, anti-bacterial, anti-oxidant, and hypoglycemic activities [10].

A previous study reported that the crude polysaccharide (PSR) from *S. rugosoannulata* exhibits immunomodulatory activity [11], indicating the promising immunomodulatory effects of PSRs. Our preliminary study also indicated that the crude PSR promoted the phagocytosis by RAW 264.7 cells. These findings suggest that PSRs may increase the immunomodulatory activity. However, the immunomodulatory PSR fractions require further exploration and the bioactive polysaccharide components need to be identified. In this study, a water-soluble purified α -D-glucan named PSRa-2 was prepared from *S. rugosoannulata* and characterized by FT-IR, HPAEC, SEM, SEC-MALLS-RI, methylation analysis, and NMR analysis. The immunomodulatory activity of this PSRa-2 was evaluated by determining the proliferation of splenocytes, and the cytokine levels. The results suggest the potential role of PSRa-2 as a functional food ingredient.

Materials and methods

Materials

Stropharia rugosoannulata was collected in November 2022 from the farm of Quzhou Gule Agricultural Development Co., Ltd., Quzhou, Zhejiang province (China). The mushroom was dried in an oven at 60 °C (Suzhou Taishuo Electric Equipment Manufacturing Co., LTD, Jiangsu, China). The dried mushrooms were blended and sieved for further use. All other chemicals and solvents were of analytically pure grade. The splenocytes of mice were purchased from Shanghai Yubo Biotechnology Co., Ltd, Shanghai, China. The kits for interleukin-2 (IL-2) and tumor necrosis factor alpha (TNF- α) were obtained from Shanghai Yuanye Bio-Technology Co., Ltd., China. All other reagents were purchased from Baoman Chemical (Shanghai, China).

Extraction of PSRs

The high pressure homogenisation-assisted enzyme method was used to extract the PSRs. Mushroom powder (500 g) was mixed with 1500 mL distilled water and the pH was adjusted to 4.5. Cellulose (1%, w/w) was used to hydrolyze the mixture at 55 °C for 1 h. The mixture was homogenized at a pressure of 20 MPa (105 °C for 0.5 h), centrifuged (10,000 \times g, 15 min) and concentrated, and then mixed with 95% ethanol (1:4, v/v), and followed by incubation at 4 °C for 24 h. The resulting precipitate was centrifuged (6000 \times g, 10 min), redissolved in distilled water, and deproteinated via Sevag method (*n*-butanol:chloroform=1:4, v/v) for eleven times. The resulting solution was dialysed and lyophilized to obtain the crude PSR.

Purification of PSR

The crude PSR was first purified through a DEAE-52 cellulose column (2.6 \times 60 cm) using H₂O and different concentrations of NaCl solutions (0–0.5 M) at 1.0 mL/min. The fractions were collected. The carbohydrate content of the eluates was determined using the phenol–sulfuric acid method [12]. The first fraction (PSRa) was further purified using a Sephacryl S-400HR column (1.6 \times 100 cm). The second fraction (PSRa-2) was collected, dialysed and freeze-dried for further use.

SEM analysis

A scanning electronic microscope (Zeiss Merlin Compact, Germany) was used to analyse the morphology of PSRa-2. The samples were coated with a thin gold layer and placed on the substrate, and observed at a voltage of 1.0 kV under high vacuum.

Structural analysis of the purified polysaccharide

A spectrometer (Nicolet iZ-10, Thermo Nicolet, USA) was used to determine the FT-IR spectrum of polysaccharides. The polysaccharides were mixed with KBr powder for FT-IR measurement in the range of 4000 to 400 cm⁻¹.

The monosaccharide composition was analyzed via HPAEC equipped with an anion-exchange column (Dionex, CarboPac PA-20) and a pulsed amperometric detector (PAD; Dionex ICS 5000 system). The detailed experimental procedure is described in Additional file 1: Methods S1.

The homogeneity and molecular weight of PSRa-2 were determined using SEC–MALLS-RI. The detailed experimental procedure is described in Additional file 1: Methods S2. The details of Congo red test are provided in Additional file 1: Methods S3.

The methylation of PSRa-2 was conducted according to the method described by Ji et al. [13] (see Additional file 1: Methods S4).

The PSRa-2 was dissolved in 0.5 mL D₂O (40 mg/mL). A Bruker AVANCE NEO 500 M spectrometer system (Bruker, Rheinstetten, Germany) was used to record the 1D-NMR and 2D-NMR of PSRa-2 at 25 °C.

In vitro immunomodulatory assay of PSRa-2

The immunomodulatory effect of PSRa-2 was evaluated based on splenocyte proliferation and protection against 5-Fu-induced immunosuppression. The splenocyte culture was performed as described previously [14]. The cytokines (TNF- α and IL-2) in the culture supernatants were measured via ELISA. The experimental procedure is described in Additional file 1: Methods S5.

Results and discussion

Isolation of PSRa-2

The elution curve of crude PSR on cellulose DEAE-52 is presented in Fig. 1a. The fraction eluted with water (PSRa) was collected for further isolation. PSRa was further purified using a Sephacryl S-400 HR column to obtain the second fraction PSRa-2. PSRa-2 was then used for structural analysis and determination of immunomodulatory activity.

Characterization of PSRa-2

SEM is a useful tool for elucidating the surface morphology of polysaccharide molecules. SEM results indicated that PSRa-2 was porous, with a loose network structure (Fig. 1b). Similarly, the fucoidan isolated from the sea cucumber *Thelenota ananas* showed a loose network structure [15].

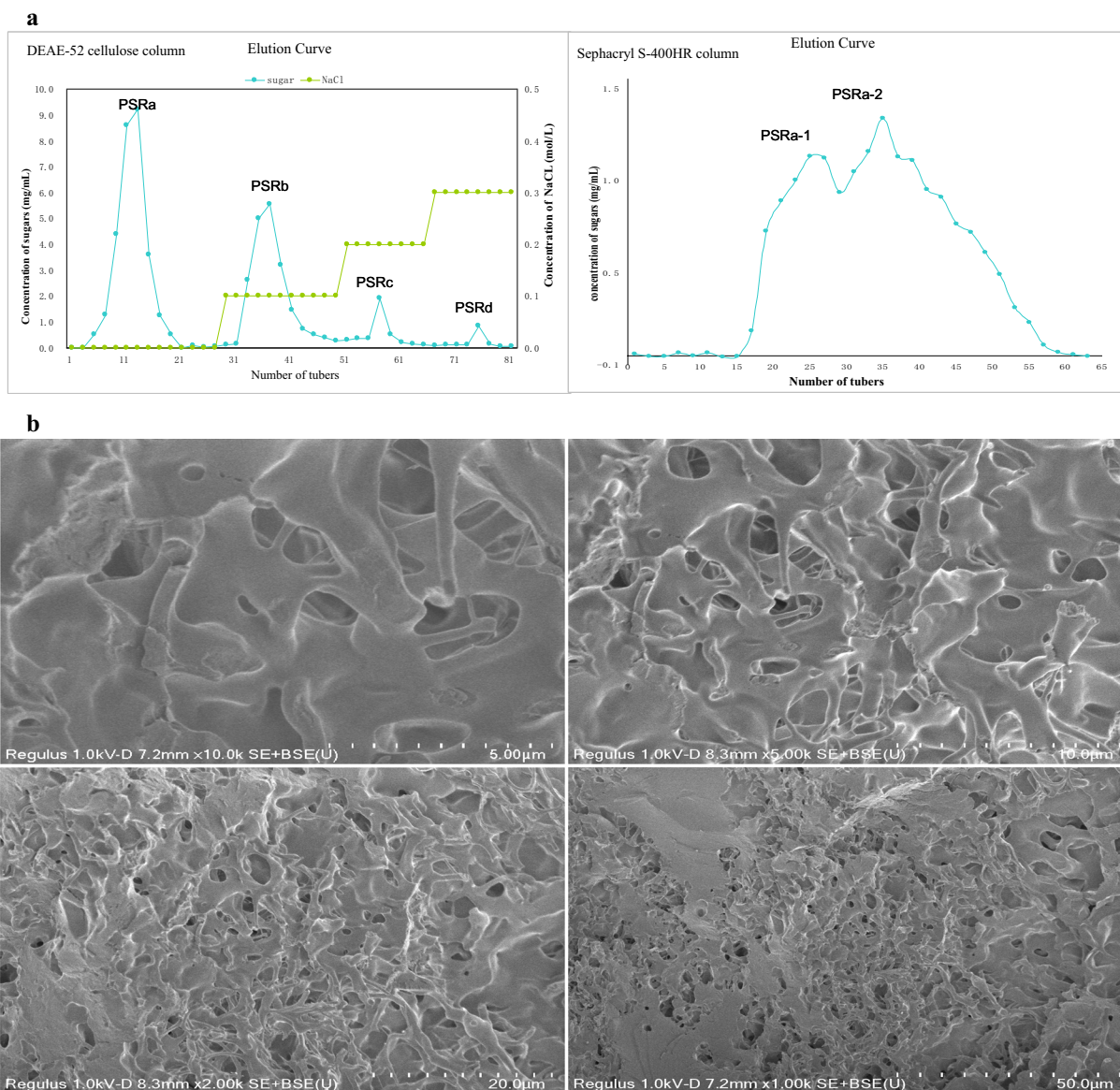


Fig. 1 a Elution curve of crude PSR on DEAE-52 cellulose and sephacryl S-400HR chromatography column; b SEM of the purified PSRa-2 (10000x, 5000x, 2000x and 1000x)

FT-IR spectra of PSRa-2 are presented in Fig. 2a. The large broadband at 3600–3200 cm^{-1} confirms the existence of a hydroxyl group, indicating the strong interaction between polysaccharide chains. The stretching vibration of C–H and C=O resulted in intense vibration peaks at 2926 cm^{-1} and 1640 cm^{-1} , respectively. The peaks ranging from 1384 cm^{-1} to 1021 cm^{-1} are attributed to the asymmetric stretching vibration of C–O. Additionally, the C–O–C stretching of α -glucosidic linkages is associated with the peaks at 848 cm^{-1} and 922 cm^{-1} [16]. The absorption at 1018 cm^{-1} , and 1076 cm^{-1} suggests an α configuration in PSRa-2 [17].

The broad peaks at 1018 cm^{-1} and 1162 cm^{-1} further confirm the presence of (1 \rightarrow 4)- α -glycosidic bonds [18].

Monosaccharide composition analysis revealed the presence of glucose alone in PSRa-2 (Fig. 2b). Similarly, a few polysaccharides derived from *Lentinus edodes*, *Grifola frondosa* and *Ganoderma lucidum* were also reported as α -glucan [19–21].

The results of HPSEC–MALLS–RI reveal PSRa-2 as a homogeneous polysaccharide with a single peak (Fig. 2c). The Mw of PSRa-2 was 455.6 kDa, which was consistent with that of fungal polysaccharides with Mw values ranging from 3.0 to 3000 kDa [22].

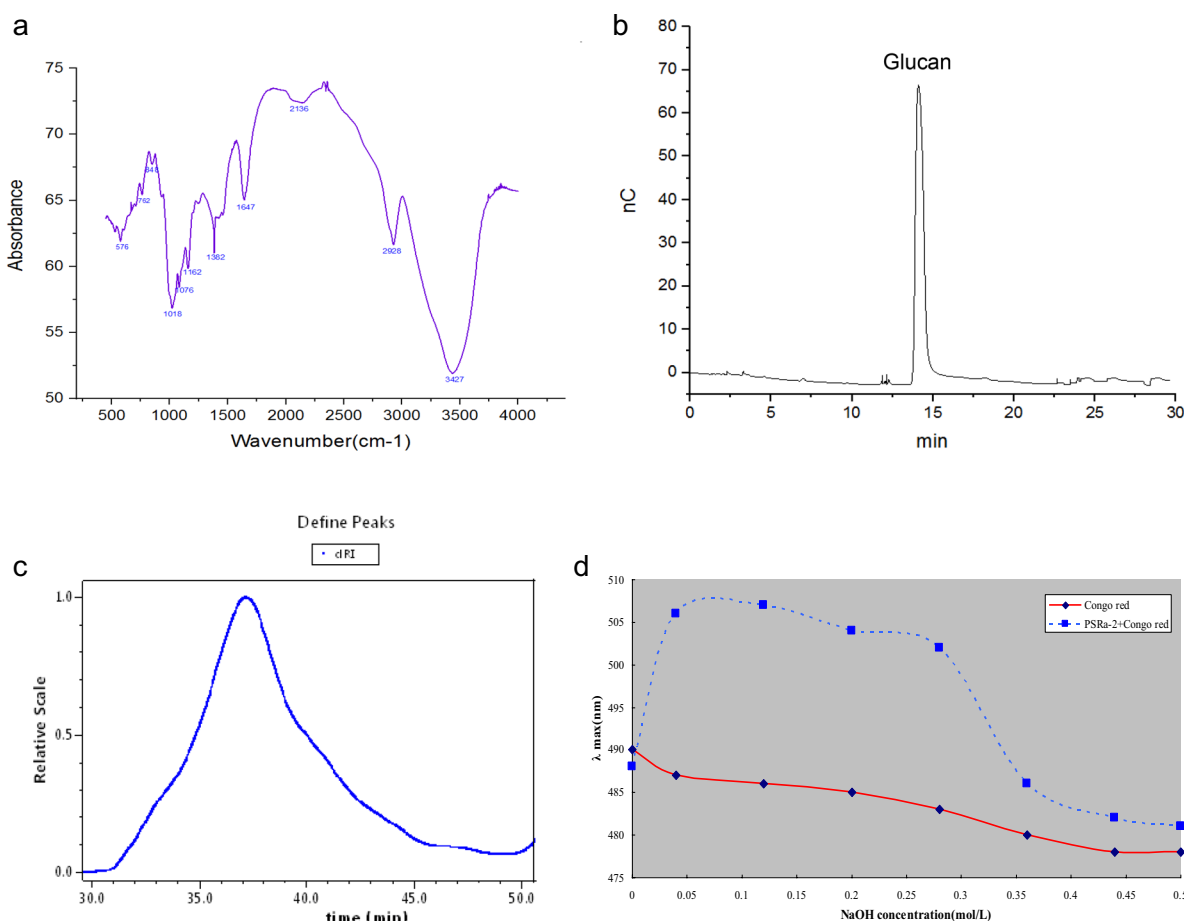


Fig. 2 FT-IR (a), monosaccharide composition (b), molecule weight (c) and Congo red test (d) of PSRa-2

Congo red test was used to test the conformation of PSRa-2. As shown in Fig. 2d, the formed complex of PSRa-2 and Congo red in NaOH solution (0–0.5 mol/L) exhibited a maximum absorbance at 507 nm and then decreased with increasing NaOH concentration, indicating that PSRa-2 carried a triple-helix structure.

The glycosidic bonds in PSRa-2 were determined by methylation and GC–MS. The sugar residues of PSRa-2 are summarized in Table 1. Six derivatives were identified

in the PSRa-2 methylation products. Among these monosaccharide residues, 4-Glc_p occupied the largest proportion of the total sugar residues (63.97%), suggesting a 4-linked Glc_p backbone of PSRa-2.

The chemical structure of PSRa-2 was further analyzed via ¹H NMR, ¹³C NMR, COSY, NOESY, HSQC, and HMBC spectroscopy. The ¹H spectrum (Fig. 3a) revealed a prominent 3.0–5.5 ppm anomeric region. The coupling signal peaks were identified in the heterotopic

Table 1 The PMAAs derived from PSRa-2

Retention time(min)	Linkage	PMAAs	Molecular weight	Molar ratios
8.12	t-Glc(p)	1,5-di-O-acetyl-2,3,4,6-tetra-O-methyl glucitol	323	10.09
11.29	3-Glc(p)	1,3,5-tri-O-acetyl-2,4,6-tri-O-methyl glucitol	351	11.71
13.17	4-Glc(p)	1,4,5-tri-O-acetyl-2,3,6-tri-O-methyl glucitol	351	63.97
15.29	3,4-Glc(p)	1,3,4,5-tetra-O-acetyl-2,6-di-O-methyl glucitol	379	1.43
16.77	3,6-Glc(p)	1,3,5,6-tetra-O-acetyl-2,4-di-O-methyl glucitol	379	3.14
17.35	4,6-Glc(p)	1,4,5,6-tetra-O-acetyl-2,3-di-O-methyl glucitol	379	9.66

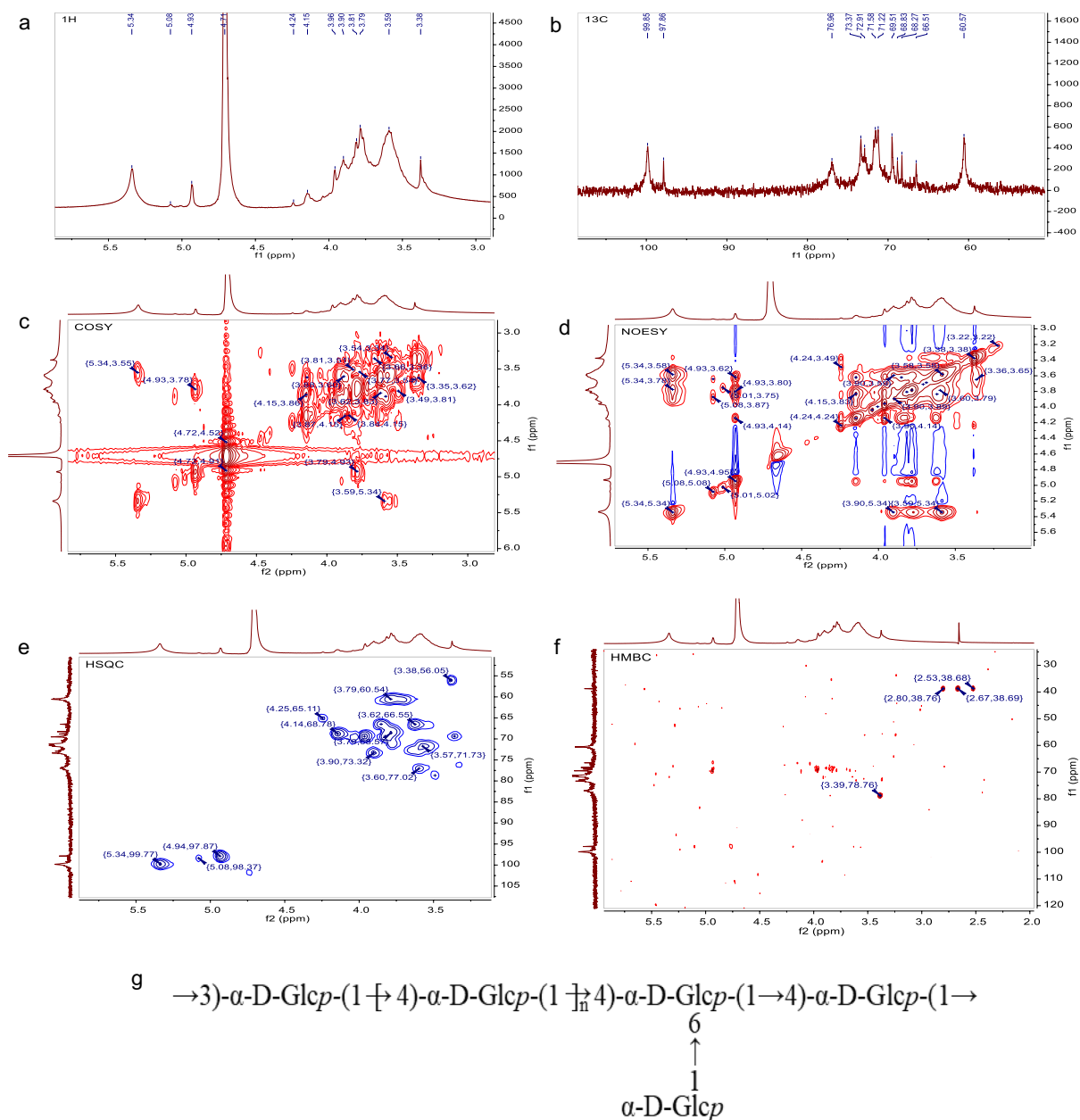


Fig. 3 The ¹H-NMR (a), ¹³C NMR (b), ¹H-¹H COSY (c), NOESY (d), HSQC (e), and HMBC (f) spectra of PSRa-2. The predicted structure of PSRa-2 (g)

signal area 4.3–5.8 ppm, which indicated the presence of several sugar residues in PSRa-2. The corresponding chemical shifts of the sugar residues were 5.34, 5.31, 5.08, and 4.93 ppm. The heterocyclic hydrogen signal of α-glycoside bond configuration was mainly distributed in the signal area of 4.8–5.8 ppm [23].

¹³C NMR spectroscopy was used to elucidate the anomeric configuration of each residue in PSRa-2 (Fig. 3b). The chemical shifts of the anomeric carbons ranged from 95 to 110 ppm. Combined with the cross peaks of

¹³C NMR and HSQC heterotopic region, the anomeric carbon signals (δ 5.34/99.77, δ 4.93/97.87, δ 5.08/98.37 and δ 5.31/99.87 ppm) of the residues were identified. The corresponding residues were →4)-α-D-Glcp-(1→, →3)-α-D-Glcp-(1→, α-D-Glcp-(1→, and →4,6)-α-D-Glcp-(1→, respectively. The chemical shifts of these glycosidic linkages in PSRa-2 were assigned and are listed in Table 2.

Based on the chemical shifts of all the sugar residues (¹³C and ¹H) and in combination with HMBC and

Table 2 ^1H and ^{13}C NMR chemical shifts of PSRa-2

Code	Glycosyl residues	Chemical shifts (ppm)					
		H1/C1	H2/C2	H3/C3	H4/C4	H5/C5	H6/C6
A	→4)-α-D-Glcp-(1→	5.34	3.55	3.96	3.59	3.89	3.79, 3.61
		99.77	71.65	69.57	77.05	73.36	60.54
B	→3)-α-D-Glcp-(1→	4.93	3.78	3.49	3.64	3.53	3.85, 3.71
		97.87	68.3	78.69	72.68	67.28	60.57
C	α-D-Glcp-(1→	5.08	3.89	3.77	3.36	3.82	3.77
		98.37	73.36	71.31	69.45	69.7	60.48
D	→4,6)-α-D-Glcp-(1→	5.31	3.57	3.95	3.58	3.9	3.85, 3.64
		99.87	71.87	69.45	77.18	73.36	66.56

NOESY results, the linkage sites and sequences of different sugar residues in PSRa-2 were analyzed (Fig. 3c–f). Because of the weak cross-peak signals in HMBC spectrum, the linkage order of residues in the polysaccharide was determined mainly from NOESY spectrum. A cross peak was detected at δ 5.34/3.59 ppm between residues A-H1 and A-H4, and a cross peak δ 5.34/3.58 ppm between residues A-H1 and D-H4. A cross peak was detected at δ 4.93/3.59 ppm between residues B-H1 and A-H4. A cross peak was found at δ 5.08/3.64 ppm between residues C-H1 and D-H6, and a cross peak δ 5.08/3.64 ppm existed between residues D-H1 and A-H4. Based on one-dimensional and two-dimensional NMR, the linkage sites and sequences of different sugar residues in PSRa-2 and the primary structure of PSRa-2 were deduced. As shown in Fig. 3g, the backbone of PSRa-2 was composed of →4)-α-D-Glcp-(1→ and →3)-α-D-Glcp-(1→ and branches of α-D-Glcp-(1→ at position of O-6 of →4,6)-α-D-Glcp-(1→.

Immunomodulatory activity of PSRa-2

Activation effect of PSRa-2 on splenocytes

As shown in Fig. 4a, compared with the control group, PSRa-2 increased the viability of splenocytes in a dose-dependent manner under the tested concentrations. Treatment with PSRa-2 doses of 200 μg/mL, 400 μg/mL and 800 μg/mL led to a remarkable increase in the cell viability to $124.5 \pm 3.59\%$, $127.4 \pm 4.21\%$ and $131.9 \pm 3.99\%$, respectively. This result indicated that PSRa-2 promoted the proliferation of splenocytes.

Effect of PSRa-2 on 5-Fu-treated splenocytes

As shown in Fig. 4b, compared with the normal control group, 5-Fu (20 μg/mL) treatment markedly decreased the proliferation of splenocytes. Conversely, the splenocytes proliferation was significantly restored by treatment

with a combination of 5-Fu and PSRa-2. The degree of recovery was increased with the dose of polysaccharides.

Effects of PSRa-2 on TNF-α and IL-2 secretion

Cytokines are signaling molecules that regulate cell differentiation, proliferation, and apoptosis to ensure homeostasis [24]. To further test the immunomodulatory activity of PSRa-2, TNF-α and IL-2 levels in the supernatants of cell cultures of splenocytes were determined. As shown in Fig. 4c and d, 5-Fu strongly inhibited the effect on TNF-α (1218.4 ± 48.3 ng/L) and IL-2 (852.4 ± 19.6 ng/L) secretion compared with the normal control group (2435.4 ± 55.3 ng/L and 1080.9 ± 28.5 ng/L, respectively). However, a dose-dependent increase of TNF-α secretion was found in the groups treated with the mixture of 5-Fu and PSRa-2. PSRa-2 significantly activated the secretion of TNF-α ($\geq 1684.5 \pm 63.3$ ng/L) at higher concentrations (> 200 μg/mL). A similar increase in IL-2 secretion was observed in the supernatant of splenocyte cultures after treatment with the combination of 5-Fu and PSRa-2. These results indicated that PSRa-2 played an immunomodulatory role by promoting the secretion of TNF-α and IL-2.

Natural polysaccharides, especially glucans, can be used as broad-spectrum agents to enhance immune regulation in the body [25]. Apart from β-glucans, various α-glucans with immune activity have also been reported. Different α-glucans may exhibit different immune mechanisms. For example, an α-glucan derived from *Pseudallescheria boydii* induces cytokine secretion via a TLR2-mediated mechanism [26]. DC-SIGN which is a C-type lectin receptors participates in the activation of immunocytes by an α-glucan isolated from *M. tuberculosis* [27]. An α-D-glucan from *Phoma herbarum* YS4108 induces B cell proliferation and activation through interacting with TLR2 and TLR4 [28] and an α-glucan derived from *Polygonum multiflorum* enhances splenocyte viability, as well as activated macrophages [29].

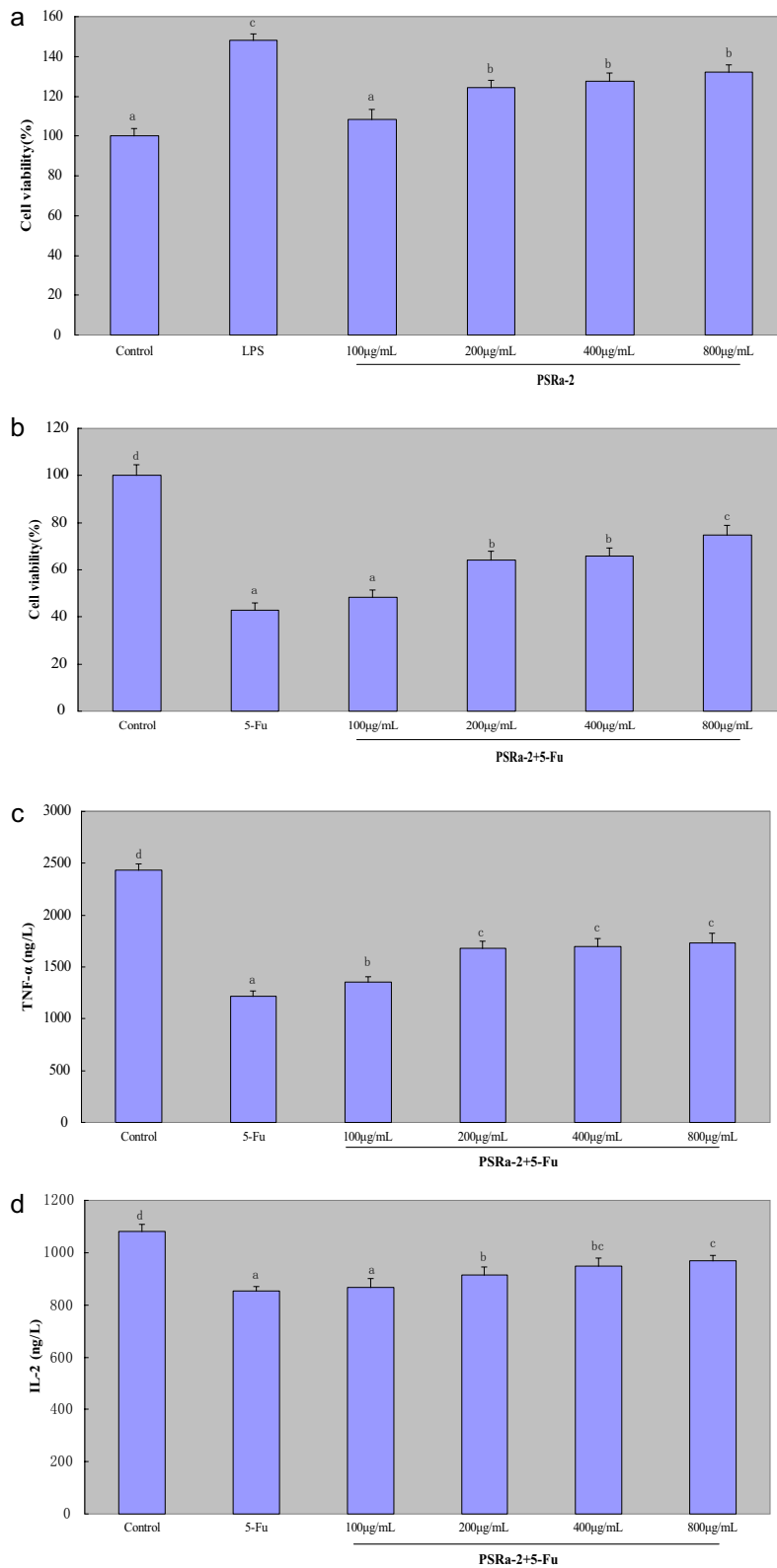


Fig. 4 Effect of different concentrations of PSRa-2 on proliferation of splenocytes (a); Cytoprotective effects of PSRa-2 on 5-Fu treated splenocytes (b); effects of the PSRa-2 on splenocyte TNF-α (c) and IL-2 (d) production. The result represented the mean ± SD (n=3). Bars without the same superscripts (a–d) denote significant difference (p < 0.05)

Splenocytes include several cell types such as dendritic cells, T and B lymphocytes, and macrophages with multiple immune functions [30]. The proliferation of splenocytes promotes cytokine secretion and eventually activates the immune response [31]. Therefore, the proliferation of splenocytes can be used as a parameter to evaluate the potential immunomodulatory activity in vitro. In this study, PSRa-2 induced immunomodulatory activity by promoting the proliferation of splenocytes. 5-Fu is a representative anticancer agent, however, it is associated with immunosuppression [32]. In this study, PSRa-2 ameliorated 5-Fu-induced cellular damage and inhibition of splenocytes. Immunosuppressants down-regulate the secretion of cytokines and the cytokine profile reflects the health of the immune system [33]. Cytokines, secreted by immune cells, strongly regulate the cell behavior. Previous studies have reported that cytokines play a significant role in immune responses, inflammation, infection, cancer and sepsis. TNF- α plays an important role in the immune system and inflammatory response [34]. IL-2 stimulates B lymphocyte proliferation and differentiation, increases T cell activity, and promotes NK cell differentiation [35]. In this study, PSRa-2 exhibited significantly activated the expression of TNF- α and IL-2 indicating the immunomodulatory effects of this α -D-glucan.

Immunostimulatory activity of an α -glucan is affected by its structural features (molecular weight, branching degree, and glycosidic linkages) [36]. It was reported that the triple helices in D-glucan are positively associated with immunostimulatory activity [37]. Previous studies have confirmed that polysaccharides rich in glucose are more likely to be recognized by membrane surface receptors [38]. The glycosidic linkages may be an important factor underlying the immunostimulatory activity of α -D-glucan. A 1, 4-linked α -D-Glcp backbone substituted at C-6 with a 1-linked D-Glcp residue from *Volvariella volvacea* significantly promoted the production of TNF- α , IL-1 β , IL-6, and NO [23]. An α -(1 \rightarrow 6)-D-glucan derived from *Armillariella tabescens* significantly increased the transcription of TNF- α , IL-1 β and IL-6 in BALB/c albino mice [39]. An α -(1 \rightarrow 4)-D-glucan from *Ophiocordyceps sinensis* enhanced the production of TNF- α , IL-6, IL-1 β , and NO [40]. SCP-1, an α -glucan composed of an α -(1 \rightarrow 4)-linked backbone with a side chain of α -(1 \rightarrow 6)-linked residue, could strengthened the phagocytosis and the secretion of cytokines by macrophages [41]. An (1 \rightarrow 3)-, (1 \rightarrow 4)- α -glucan from *Polyporus grammoccephalus* modulated the immune system [42]. (1 \rightarrow 4)-, (1 \rightarrow 6)- α -D-glucans derived from *Agaricus blazei* and *Tricholoma matsutake* have been reported to possess immunomodulating and antitumor activities [43, 44]. A branched (1 \rightarrow 3)- α -D-glucan, isolated from spores

of *Ganoderma lucidum*, was reported to enhance lymphocyte proliferation and antibody production [45]. In this study, PSRa-2 composed of \rightarrow 4)- α -D-Glcp-(1 \rightarrow and \rightarrow 3)- α -D-Glcp-(1 and branched of α -D-Glcp-(1 \rightarrow at position of O-6 of \rightarrow 4,6)- α -D-Glcp-(1 \rightarrow exhibited significant immunomodulatory activity by promoting proliferation of splenocytes and protecting immunocytes against 5-Fu-induced immunosuppression. Branching pattern and three-dimensional conformations and molecular weight may be positively factors on its immunomodulatory activity.

Conclusion

In this study, a novel polysaccharide named PSRa-2 was purified from the fruiting body of *Stropharia rugosoannulata* and its structural characteristics and immunomodulatory activities were investigated. PSRa-2 is an alpha-glucan with a Mw of 455.6 kDa. PSRa-2 is composed of \rightarrow 4)- α -D-Glcp-(1 \rightarrow and \rightarrow 3)- α -D-Glcp-(1 \rightarrow and branched of α -D-Glcp-(1 \rightarrow at position of O-6 of \rightarrow 4,6)- α -D-Glcp-(1 \rightarrow . PSRa-2 exhibits significant immunomodulatory activity by promoting the proliferation of splenocytes and protecting immunocytes against 5-Fu-induced immunosuppression. Branching pattern, three-dimensional conformation and molecular weight may have strongly effects on its immunomodulatory activity.

Abbreviations

FT-IR	Fourier Transform infrared spectra
HPAEC	High-performance anion-exchange chromatography
SEM	Scanning electronic microscope
SEC-MALLS-RI	Size-exclusion chromatography–multiangle laser light scattering-differential refractive index detectors
NMR	Nuclear magnetic resonance
GC-MS	Gas chromatography–mass spectrometry

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s40538-023-00475-8>.

Additional file 1: Methods S1. Monosaccharide composition analysis of PSRa-2. Approximately 5 mg of sample was hydrolyzed with trifluoroacetic acid (2 M) at 121 °C for 2 h in a sealed tube. Dry the sample with nitrogen. Add methanol to wash, then blow dry, repeat methanol, and wash 2–3 times. The residue was re-dissolved in deionized water and filtered through 0.22 μ m microporous filtering film for measurement. The sample extracts were analyzed by high-performance anion-exchange chromatography (HPAEC) on a CarboPac PA-20 anion-exchange column (3-by-150 mm; Dionex) using a pulsed amperometric detector (PAD; Dionex ICS 5000 system) by Sanshu Biotech. Co., LTD (Shanghai, China). Flow rate, 0.5 mL/min; injection volume, 5 μ L; solvent system A: (ddH₂O), solvent system B: (0.1 M NaOH), solvent system C: (0.1 M NaOH, 0.2 M NaAc); gradient program, volume ratio of solution A, B, C was 95:5:0 at 0 min, 85:5:10 at 26 min, 85:5:10 at 42 min, 60:0:40 at 42.1 min, 60:40:0 at 52 min, 95:5:0 at 52.1 min, and 95:5:0 at 60 min. Data were acquired on the ICS5000 (Thermo Scientific), and processed using chromeleon 7.2 CDS (Thermo Scientific). **Methods S2.** Homogeneity and molecular weight determination. The samples were dissolved in 0.1 M NaNO₃ aqueous

solution containing 0.02% NaNO₃ at the concentration of 1 mg/mL and filtered through a filter of 0.45 μm pore size. The samples were dissolved in DMSO solution containing lithium bromide (0.5% w/w) (DMSO/LiBr) at the concentration of 1 mg/mL and filtered through a filter of 0.45 μm pore size. The homogeneity and molecular weight of various fractions were measured using SEC-MALLS-RI. The weight and number-average molecular weight (M_w and M_n) and polydispersity index (M_w/M_n) of various fractions in 0.1 M NaNO₃ aqueous solution containing 0.02% NaNO₃ (or DMSO solution containing 0.5% LiBr) were measured on a DAWN HELEOS-II laser photometer Wyatt Technology Co., USA) equipped with Three tandem columns (300 × 8 mm, Shodex OH-pak SB-805, 804 and 803; Showa Denko K.K., Tokyo, Japan) which was held at 45 °C (or 60 °C) using a model column heater by Sanshu Biotech. Co., LTD (Shanghai, China). The flow rate is 0.5 mL/min (or 0.3 mL/min). A differential refractive index detector (Optilab T-REX, Wyatt Technology Co., USA) was simultaneously connected to give the concentration of fractions and the dn/dc value. The dn/dc value of the fractions in 0.1 M NaNO₃ aqueous solution containing 0.02% NaNO₃ was determined to be 0.141 mL/g, and in DMSO solution was determined to be 0.07 mL/g. Data were acquired and processed using ASTRA6.1 (Wyatt Technology). **Methods S3.** Congo red test. The conformation of PSRa-2 was determined by the Congo red test. Various concentrations of NaOH were added to a mixture of PSRa-2 (2 mg/mL) and Congo red (80 μM) and the mixtures were left to stand for 10 min. The maximum absorption wavelength of the samples was recorded using a spectrophotometer (Shanghai Precision and Scientific Instrument Co., Ltd, Shanghai, China). **Methods S4.** The methylation analysis of PSRa-2. The polysaccharide sample was dissolved in DMSO. The solutions were methylated in DMSO/NaOH with CH₃I. After complete methylation, the permethylated products were hydrolyzed with 2 mol/L TFA at 121 °C for 1.5 h, reduced by NaBD₄ and acetylated with acetic anhydride for 2.5 h (100 °C). The acetates were dissolved in chloroform and analyzed with GC-MS on an Agilent 6890A-5975C equipped with Agilent BPX70 chromatographic column (30 m × 0.25 mm × 0.25 μm, SGE, Australia), and high purity helium (split ratio 10:1) was used as the carrier gas with an injection volume of 1 μL by Sanshu Biotech. Co., LTD (Shanghai, China). Mass spectrometry analysis was performed at the initial temperature of 140 °C for 2.0 min, and the temperature is increased to 230 °C by 3 °C/min for 3 min. **Methods S5.** Immunomodulatory effect of PSRa-2 on the splenocytes of mice. The potential immunomodulatory effect of PSRa-2 was evaluated by determining its potential activities on proliferation of splenocytes and protection on spleocytes against 5-Fu induced immunosuppression. Briefly, the aqueous treatment solution of PSRa-2 was first prepared in serial concentrations. Splenocytes were cultured in 96-well plates for 24 h, respectively. After incubated with treatment solution of PSRa-2 for another 48 h, the cell viability was measured based on MTT method, and the absorbance at 570 nm was obtained to calculate the cell viability of splenocytes. The cytokines (TNF-α and IL-2) exist in the culture supernatants of splenocytes were measured by ELISA kits according to the manufactures' manner. All experiments were performed in triplicate.

Acknowledgements

The authors are grateful to Shanghai Sanshu Biotechnology Co., LTD for their help in the purified polysaccharide.

Author contributions

Conceptualization and methodology: ZZ; writing original draft preparation: ST; visualization and supervision: LG; software and validation: LJ; investigation and resource: JQ.

Funding

This research was founded by the New Variety Breeding Project of Science Technology Department of Zhejiang Province (2021C02073) and supported by the China Agriculture Research System CARS-20.

Availability of data and materials

The datasets used during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interest.

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Received: 28 July 2023 Accepted: 17 September 2023

Published online: 25 September 2023

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