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Structure and immunobiological activity of a new polysaccharide from *Bletilla striata*

Qiang Peng[a,*], Ming Li[c], Feng Xue[a], Huajing Liu[b,**]

[a] College of Food Science and Engineering, Northwest A&F University, YangLing 712100, China
[b] College of Resources and Environmental Sciences, Northeast Agricultural University, Harbin 150030, China
[c] College of Resources and Environment, Northwest A&F University, YangLing 712100, China

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ABSTRACT

A new polysaccharide (designated BSPF2), with a molecular weight of 2.35×10^5 Da, was isolated from the tubers of *Bletilla striata*. It contained mannose, glucose, and galactose in a molar ratio of 9.4:2.6:1.0. The acetyl content of BSPF2 was estimated to be 2.9%, and acetyl groups were located in positions 3 and 6 of mannosyl residues. The structural features were elucidated by a combination of monosaccharide composition analysis, periodate oxidation, partial acid hydrolysis, acetolysis, and methylation analysis. The results indicated that the backbone of BSPF2 consisted of (1 → 4)-linked mannosyl residues and (1 → 4)-linked glucosyl residues in a molar ratio of 2:1. About three fifths of glucosyl residues in the backbone were branched at O-6 position, and the terminal sugar residues were composed of mannosyl residues. Immunological assay results demonstrated that BSPF2 significantly induced the spleen cell proliferation in a dose-dependent manner.

1. Introduction

Bletilla striata (Thunb.) Reichb.f. belongs to the Orchid family. It is mainly distributed in China, North Korea, Japan and Burma. The tubers of *B. striata* is a well-known traditional Chinese herb and is used to treat swollen tissues, skin cracks, freckles, burns and abscesses. Pharmacological researches have shown that herbal polysaccharides are an important functional factor in traditional Chinese medicine. The tubers of *B. striata* contains a lot of polysaccharide. It was reported that *B. striata* polysaccharide could induce the proliferation of human umbilical vascular endothelial cells and vascular endothelial growth factor (Wang et al., 2006). Furthermore, it was also found that *B. striata* polysaccharide could act on macrophage and simulate cells to expression some proinflammatory cytokines (Diao et al., 2008). However, there are little reports on the structural features of *B. striata* polysaccharide. An early publication reported that a *B. striata* polysaccharide contained mannose and glucose in a molar ratio of 3:1, with the backbone consisting of (1 → 4)-linked aldohexopyranosyl residues (Tomoda, Nakatsuka, Tamai & Nagata, 1973). Another *B. striata* polysaccharide was reported to be composed of mannose and glucose in a molar ratio of 2.4:1.0, with the molecular weight of 1.35×10^5 Da (Wang et al., 2006). These results indicated that *B. striata* polysaccharide contain more than one kind of fractions.

In the present study, a novel polysaccharide fraction (BSPF2) was isolated from the tubers of *B. striata*. The structural features of BSPF2 was elucidated by monosaccharide composition, periodate oxidation, partial acid hydrolysis, acetolysis, and methylation analysis. Further, the immunobiological activity of BSPF2 was analyzed by ^{3}H-thymidine incorporation method.

2. Materials and methods

2.1. Plant material

The tubers of *Bletilla striata* was purchased from Huike Botanical Development Cooperation, PR China. The plant material was oven-dried at 60 °C and subsequently crushed into powder.

2.2. Chemicals

Standard monosaccharides and T-dextran series of different standard molecular weights were purchased from Sigma Chemical Co. (USA); DEAE-Cellulose-52 was purchased from Yuanye biological technology Co. (Shanghai, China); Bio-gel-P-300 was purchased

* Corresponding author. Tel.: +86 29 87091746; fax: +86 29 87091746.
** Corresponding author. Tel.: +86 451 55191137; fax: +86 451 55190720.
E-mail addresses: pengqiang@nwsuaf.edu.cn, pengqiang3931@163.com (Q. Peng), lileaf@163.com (M. Li), xuefengscholar@126.com (F. Xue), huajingliu@neau.edu.cn (H. Liu).

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from Bio-Rad Co. (USA); Dowex 50 WX8-400 cation exchange resin was purchased from Sigma–Aldrich (St. Louis, MO, USA); LPS (*Escherichia coli* 055:B5) was purchased from Sigma–Aldrich (St. Louis, MO); other reagents used were of analytical grade and supplied by Sinopharm Chemical Reagent Ltd. Co., (Shanghai, China).

2.3. Analytical methods

The carbohydrate content was determined by the phenol-sulfuric acid method using dextran as a standard (Dubois, Gilles, Hamilton, Rebers & Smith, 1956). The protein content was estimated by the method of Bradford using ovalbumin as a standard (Bradford, 1976). A UV scan in the region of 200–400 nm was performed on the spectrophotometer. Starch contamination was estimated by treatment with pancreatic α-amylase (Asamizu & Nishi, 1979). The homogeneity and molecular weight of samples were determined by high performance gel permeation chromatography on a TSK-Gel G4000SW column, using a Waters Alliance 2414 refractive index detector (Alsop & Vlachogiannis, 1982). Acetyl groups were located according to the method of De-Belder (De Belder & Norrman, 1969).

2.4. Isolation and purification of B. striata polysaccharide

The *B. striata* tubers powders (1000 g) was extracted with acetone at 50 °C for 4 h. After filtration, the residue was suspended in 5000 mL distilled water and extracted for 2 h at 80 °C. After suction filtration, the extraction was similarly repeated. All extraction solutions were pooled and concentrated with a rotary evaporator at 40 °C. A 4-fold volume of ethanol was added to the extracts. The precipitate was treated with Sevag reagent (Navarini et al., 1999) to remove proteins and dialyzed against distilled water for 48 h. After lyophilization, crude *B. striata* polysaccharide (CBSP) was obtained. CBSP was dissolved in distilled water at 45 °C with continuous stirring and the solution was adjusted to pH 9.0 with aqueous ammonia. After stirring for another 4 h, the solution was neutralized with 1 mol/L HCl followed by dialysis and lyophilization. This decolorized CBSP (1.0 g) was dissolved in distilled water and applied to a anion-exchange column (6.0 cm × 55.0 cm) of DEAE-Cellulose-52. The column was first washed with distilled water, and then eluted with a step gradient (0.05, 0.10 and 0.15 mol/L NaCl) at a flow rate of 1 mL/min. Sugar-positive fractions were combined, concentrated, dialyzed against distilled water, and lyophilized. The obtained fractions were designated as BSP1, BSP2, BSP3 and BSP4, respectively. BSP2 (80 mg) was further purified by gel permeation chromatography on a Bio-Gel P-300 column (1.5 cm × 100 cm) using 0.1 mol/L NaCl solution as an eluent. The fractions corresponding to the main carbohydrate-containing peak of the chromatography elution profile were pooled. Then the purified polysaccharide BSPF2 was obtained.

2.5. Determination of monosaccharide composition

Polysaccharides (2 mg) was hydrolyzed with 2 mol/L trifluoroacetic acid (2 mL) at 120 °C for 2 h. The hydrolyzate was evaporated with a rotary evaporator. Neutral sugars and uronic acids were simultaneously detected by GC using the method described previously (Lehrfeld, 1985). GC was performed by a Shimadzu GC2010 equipped with a capillary column of rtx-5ms (30.0 m × 0.25 mm × 0.25 μm). The temperature program was: 180 °C. for 2 min, then to 210 °C. at 6 °C/min, then to 215 °C at 0.3 °C/min, then to 240 °C at 6 °C/min for 45 min N_2 was used as the carrier gas at 0.6 mL/min.

2.6. Periodic acid oxidation and Smith degradation

BSPF2 was oxidized with 0.04 M $NaIO_4$ in the dark at 4 °C until stabilization of periodate consumed (Ghosh et al., 2008; Hu, Kong, Yang & Pan, 2011). The excess periodate was destroyed by adding ethylene glycol, and the solution was dialyzed against distilled water for 2 d. After it was reduced with $NaBH_4$ at 25 °C for 12 h, the residue was subjected to complete hydrolysis with 2 mol/L trifluoroacetic acid. The acid was removed by co-distillation with methanol under vacuum. Finally, the products were acetylated and analyzed in GC by using the same method as described in Section 2.5.

2.7. Selective hydrolysis of the branched polysaccharide

BSPF2 (80 mg for each hydrolysis) was dissolved in 10 mL of 0.02 mol/L H_2SO_4 and heated at 80 °C for 8 h and 12 h (Peng et al., 2012). The resulting solution was neutralized with NaOH and dialyzed against distilled water using membranes with size exclusion 3.5 kDa, and retained polymeric samples were lyophilized, giving rise to HP8 and HP12 fractions, respectively.

BSPF2 (80 mg) was acetylated by a mixture of acetic anhydride, acetic acid and sulfuric acid (10:10:1, v/v, 10 mL) at 20 °C for 18 h (Wu, Sun & Pan, 2006). The resulting solution was neutralized with sodium hydrogen carbonate and was further purified on a Sephadex G-10 column, giving the partially acetylated polysaccharide AP1 and oligosaccharide fractions AP2. All the above samples were submitted to sugar composition analysis and methylation analysis.

2.8. Methylation analysis

Samples were methylated using modified Ciucanu method as described previously (Needs & Selvendran, 1993). The methylation procedure was repeated three times. Next, the samples were hydrolyzed with 2 mol/L trifluoroacetic acid (121 °C, 2 h), reduced with $NaBH_4$, and acetylated to convert into their partially methylated alditol acetates. The resulting alditol acetates were analyzed by GC and GC–MS. Peaks of methylated sugars were identified by their mass spectra. Their relative molar ratios were estimated from the peak areas of GC and corresponding response factors. Response factors of partially methylated and alditol acetates are calculated by the effective carbon response (Sweet, Shapiro & Albersheim, 1975). GC–MS was performed using a Shimadzu instrument GCMS-QP2010 equipped with an electron impact ion source. The capillary column used was rtx-5ms (30 m × 0.25 mm × 0.25 μm); the temperature program was: 140 °C. for 2 min, then to 250 °C. at 2 °C/min in 30 min. Helium was used as a carrier gas and the flow rate was 0.6 mL/min. The temperatures of the interface and the ion source were 200 °C and 250 °C, respectively.

2.9. Spleen cells proliferation assay

Balb/C mice were sacrificed. The spleen was aseptically removed and minced through a 40 μm nylon cell strainer to achieve single cell suspension. Red blood cells were depleted with Tris-NH_4Cl lysis buffer. 5×10^5 spleen cells were stimulated with PSPF2 at serial concentrations, including 10, 50, 100 μg/mL. LPS (5 μg/mL) was positive control for the proliferation of the spleen cells. The cells were cultured in RPMI-1640 medium, supplemented with 10% FBS, penicillin (100 U/mL) and streptomycin (100 μg/mL) at 37 °C in a 5% CO_2 humidified incubator for 72 h and were pulsed with ^{3}H-thymidine (1 μCi/well) for the last 18 h. The cells were harvested on glass fiber filters using a Filtermate cell harvester (Packard). The amount of ^{3}H-thymidine incorporated into cells was measured using a β-scintillation counter (BECKMAN LS6500). The results are

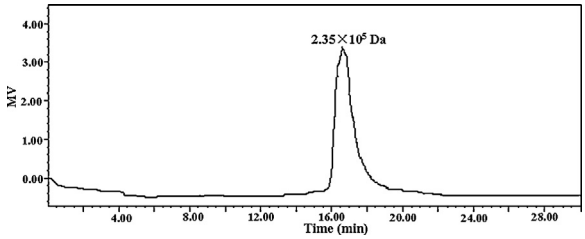

Fig. 1. Elution profile of BSPF2 on high performance liquid gel permeation chromatography.

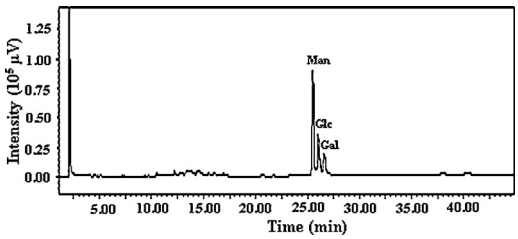

Fig. 2. Gas chromatography of alditol acetate derivatives of acid hydrolyzates of BSPF2. Column: rtx-5ms (30 m × 0.25 mm × 0.25 μm); temperature: 180 °C (2 min) – 6 °C/min, 240 °C (45 min); carrier gas: N_2 (0.6 mL/min).

expressed as cpm (counts per minute) of stimulated cells and cpm of unstimulated cells.

3. Results and discussion

3.1. Isolation of polysaccharide from B. striata tubers

For purpose of removing lipids and hydrophobic components, the *B. striata powder* was extracted with acetone, and the supernatant was removed. The residue was extracted with hot water, giving a crude polysaccharide sample, CBSP. The CBSP was obtained in a yield of 4.4% based on the dried weight of the plant powder. CBSP was composed of glucose, mannose and galactose in a molar ratio of 1.0:5.4:0.7. The carbohydrate content of CBSP was 91.3% and the protein content was 2.6%. CBSP was separated by anion exchange chromatography, which was successively eluted with H_2O, 0.05, 0.10 and 0.15 mol/L NaCl. Four polysaccharide subfractions, named as BSP1, BSP2, BSP3, and BSP4, were obtained. The component monosaccharides of BSP1 were mannose and glucose, and the molar ratio of them was 3.2:1.0, which was basically the same with previous research (Tomoda et al., 1973). BSP2 was the dominant sub-fraction accounting for 54.7% of the total polysaccharide recovered from the anion exchanger. BSP2 was further purified by gel permeation chromatography on a Bio-Gel-P 300 column. A polysaccharide fraction was obtained with a yield of 0.9% of the *B. striata tubers* and named BSPF2. The homogeneity and molecular weight of BSPF2 were determined by high-performance gel permeation chromatography. The elution profile showed a single and symmetric sharp peak (Fig. 1), indicating that BSPF2 was a homogeneous polysaccharide. The linear regression was calibrated with dextrans 10,000, 40,000, 80,000, 120,000, 200,000 and 400,000. The calibration curve equation is: $Log\ MW = 5.3089 - 2.3825X$, $R^2 = 0.9908$, in which MW denotes the molecular weight of the standard dextran and X is the retention time. According to the retention time, the molecular weight of BSPF2 was estimated to be 2.35×10^5 Da.

3.2. Characterization of polysaccharide from B. striata tubers

BSPF2 was resistant to α-amylase, thus proving the absence of starch. The total carbohydrate content of BSPF2 was 96.2% according to the phenol-sulfuric acid method; the protein was absent according to the Bradford method. Ultraviolet spectrum of BSPF2 showed that absorption peaks at 260 nm and 280 nm were not detected, further suggesting the absence of nucleic acid and protein in BSPF2. The sulfate-carbazole method showed the absence of uronic acid (Bitter & Muir, 1962). BSPF2 was hydrolyzed with trifluoroacetic acid, and the constituent monosaccharides were converted into alditol acetates. The GC chromatogram of the alditol acetates of monosaccharides was shown in Fig. 2. BSPF2 was composed of three kinds of monosaccharides, namely mannose, glucose and galactose in a molar ratio of 9.4:2.6:1.0. Thus BSPF2 had different properties from the *B. striata* polysaccharide described in the former report (Tomoda et al., 1973). Its monosaccharide composition was summarized in Table 1. The results indicated that mannose and glucose were the predominant monosaccharide, implying the presence of glucomannan. On the other hand, the acid hydrolysate of BSPF2 was analyzed directly by GC. A obvious peak was identified as acetic acid, demonstrating the presence of O-acetyl groups in BSPF2. The acetyl content of the polymer was 2.9%, and the degree substitution (DS) was 0.11 (Liu et al., 2012). In order to make clear the location of O-acetyl groups in BSPF2, it was determined by reation with methylvinyl ether, deacetylation, methylation, hydrolysis, reduction and acetylation. These derivatives contained 1,2,4,5,6-penta-O-acetyl-3-O-methyl-mannitol, 1,2,4,5-tetra-O-acetyl-3,6-di-O-methyl-mannitol, hexa-O-acetyl-mannitol, hexa-O-acetyl-glucitol and hexa-O-acetyl-galactitol in a molar ratio of 1.0:0.4:9.9:3.1:1.3. Since methyl groups in the derivatives marked the positions originally occupied by acetyl groups, the result indicated that the acetyl groups were attached to positions 3 and 6 of mannosyl residues, and approximately 12.4% of mannosyl residues had acetyl groups. The polysaccharide also contained unsubstituted glucosyl and galactosyl residues.

3.3. Structural analysis of polysaccharide from B. striata tubers

Glycosidic linkage location of polysaccharides may be preliminarily determined by consumption of $NaIO_4$ and production of HCOOH in periodic acid oxidation. BSPF2 was completely oxidized in 4 d with 0.04 M sodium periodate at 4 °C. In the periodic acid oxidation, 1 mol glycosyl consumed 1.170 mol $NaIO_4$ then produced 0.157 mol HCOOH. On account of formic acid liberation after periodate oxidation, it was indicated that the non-reducing terminal residues or (1 → 6)-linked glycosidic bonds were existed in BSPF2. The periodate-oxidized products of BSPF2 were reduced, hydrolyzed, acetylated and analyzed by GC. The glycerol and erythritol were found with the molar ratio of 1.0:5.1. Mannose, glucose and galactose were not found in the hydrolyzed products. The results indicated that mannosyl, glucosyl and galactosyl residues were in linkages that can be oxidized, namely (1 →)-linked, (1 → 2)-linked, (1 → 4)-linked, (1 → 6)-linked, (1 → 2,6)-linked or (1 → 4,6)-linked.

To obtain more information about the sugar linkages, BSPF2 was subjected to methylation analysis. After complete methylation,

Table 1
Monosaccharide composition of BSPF2 and its hydrolysates.

Fractions	Sugar composition (%)		
	Man	Glc	Gal
BSPF2	72.4	19.9	7.7
HP8	66.7	23.7	9.6
HP12	60.2	28.2	11.6
AP1	65.9	34.1	–
AP2	78.8	–	21.2

–: Not detected; Man: mannose; Glc: glucose; Gal: galactose.

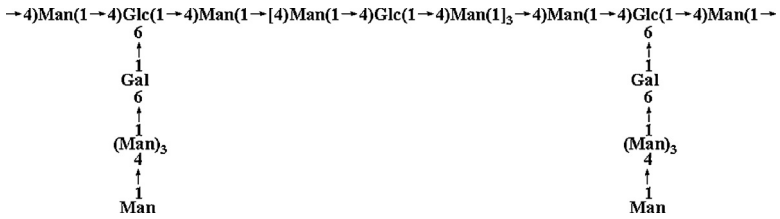

Fig. 3. The hypothetical structure of the repeat unit of BSPF2.

hydrolysis and alditol acetate derivatization, partially methylated alditol acetates were analyzed by GC and GC–MS. Based on methylation analysis (Table 2), it could be suggested that BSPF2 is a branched polysaccharide, containing non-reducing termini of mannose and branches of (1 → 4)-linked mannose, (1 → 4,6)-linked glucose, (1 → 4)-linked glucose and (1 → 6)-linked galactose. And the molar ratio of glycosyl residues was in good agreement with the results of periodate oxidation and Smith degradation. The number of branch points was equal to the number of terminal units, showing that BSPF2 was fully methylated. The methylation analysis results showed that the dominating glycosyl residues of BSPF2 were mannosyl residues and glucosyl residues, which was consistent with the result of monosaccharide composition analysis, implying that the polysaccharide was a glucomannan.

In order to elucidate the linkages between backbone and side chains of polysaccharides, partial acid hydrolysis of BSPF2 was carried out for 8 and 12 h, which gave rise to hydrolysates HP8 and HP12, respectively. The molecular weight of HP8 and HP12 were 1.91×10^5 Da and 1.59×10^5 Da, respectively. The monosaccharide composition of HP8 and HP12 were listed in Table 1. It showed that the amount of mannose decreased as the hydrolysis time increased. Compared with BSPF2, obvious increases of glucose and galactose in HP12 were observed, confirming the presence of mannose in side chains of the polysaccharide. Glucose was likely located on the backbone of BSPF2. Further detailed structural information was investigated by methylation analysis of HP8 and HP12. HP12 contained six glycosyl residues, namely terminal mannosyl, terminal galactosyl, (1 → 4)-linked mannosyl, (1 → 4)-linked glucosyl, (1 → 6)-linked galactosyl and (1 → 4,6)-linked glucosyl residues in a molar ratio of 1:1:10:3:1:2 (Table 2). HP12 had high proportions of (1 → 4)-linked mannosyl residues, (1 → 4)-linked glucosyl residues and (1 → 4,6)-linked glucosyl residues, in accord with polymers comprising a glucomannan backbone, which were substituted at the O-6 or O-4 of glucosyl residues by side chains. Compared to BSPF2, the amount of (1 → 4)-linked mannosyl and (1 → 6)-linked galactosyl residues of HP12 decreased, indicating the presence of (1 → 4)-linked mannosyl and (1 → 6)-linked galactosyl residues in side chains. With hydrolysis time extension, the new terminal galactosyl residues in HP12 were accompanied by the decrease of (1 → 6)-linked galactosyl residues, suggesting that O-6 position of galactosyl residues were substituted by (1 → 4)-linked mannosyl residues in side chains. After partial acid hydrolysis, the decrease of (1 → 6)-linked galactosyl residues was probably due to the hydrolysis at O-6 position of galactosyl residues and the conversion of (1 → 6)-linked galactosyl residues to terminal galactosyl residues. Similarly, a small number of (1 → 4,6)-linked glucosyl residues (5.6%) was identified in HP12, indicating that side chains had not been fully hydrolyzed by 0.02 mol/L H_2SO_4 at 80 °C for 12 h.

Table 2
The results of methylation analysis of BSPF2 and its hydrolysates.

Methylated sugar[a]	Deduced linkage[b]	Molar ratio			
		BSPF2	HP8	HP12	AP1
2,3,4,6-Me_4-Man	Man (1→	2	2	1	–
2,3,4,6-Me_4-Gal	Gal(1→	–	–	1	–
2,3,6-Me_3-Man	→4) Man (1→	16	12	10	10
3,6-Me_2-Glc	→2,4) Glc (1→	3	3	3	5
2,4,6-Me_3-Gal	→3) Gla (1→	2	2	1	–
2,3,6-Me_3-Glc	→4) Glc (1→	2	2	2	–

–: Not detected.

[a] Analyzed by GC–MS, after per-O-methylation, total acid hydrolysis, reduction, and acetylation. 2,3,4,6-Me_4-Man = 1,5-di-O-acetyl-2,3,4,6-tetra-O-methylmannose, etc.

[b] Based on derived O-methylalditol acetates.

After partial acetolysis of BSPF2, two fractions AP1 and AP2 were obtained by Sephadex G-10 chromatography. Based on HPLC, AP1 was a homogeneous polymer, with the molecular weight of 1.51×10^5 Da, which was not probably caused by some degradation of the backbone. The results of methylation analysis (Table 2) indicated AP1 was a linear (1 → 4)-linked glucomannan, with a high proportion of mannosyl residues (66.7%). No branched glycosyl residues were found, suggesting that side chains had been entirely cleaved by acetolysis. Partial acetolysis resulted in disappearing of (1 → 4,6)-linked glucosyl residues and the conversion of (1 → 4,6)-linked glucosyl residues to (1 → 4)-linked glucosyl residues. The result was in accordance with previous observations that (1 → 6)-linked glycosidic bond was easily cleaved by acetolysis (Rosenfeld & Ballou, 1974). Galactosyl residues was not detected in AP1, suggesting that all (1 → 6)-linked galactosyl residues were located at sidechains. These results indicated that the backbone of BSPF2 was partially substituted at O-6 of glucosyl residues by (1 → 6)-linked galactosyl residues. AP2 was identified as a mixture of oligosaccharides by ESI-MS, and it was composed of only mannose and galactose (Table 1), further confirming that (1 → 4)-linked and (1 → 4,6)-linked glucosyl residues were all in the main chain of BSPF2.

3.4. Structural model of polysaccharide from B. striata tubers

Based on the linkages analysis results of intact and partially degraded BSPF2, a hypothetical structure of an average repeating unit of BSPF2 was shown in Fig. 3. BSPF2 was made up of a backbone of (1 → 4)-linked mannosyl residues and (1 → 4)-linked glucosyl residues in a molar ratio of 2:1, three fifths of glucosyl residues in the backbone were substituted at O-6 position with side chains. Its branches were composed of (1 → 6)-linked galactosyl residues and (1 → 4)-linked mannosyl residues, and the terminal sugar residues were mannosyl residues.

3.5. Immunological activity of polysaccharide from B. striata tubers

In order to avoid false positive of the immunological test of the polysaccharides, possible LPS contamination of BSPF2 was excluded using Tachypleus amebocyte lysate assay (obtained from Chinese Horseshoe Crab Reagent Manufactory, CO., Ltd., Xiamen, China), with the LPS concentration lower than 0.1 EU/μg. To observe the immune activity of BSPF2, mouse spleen cells were stimulated with BSPF2 at 10–100 μg/mL for 72 h. In order to quantify the proliferation rate, the DNA synthesis as revealed

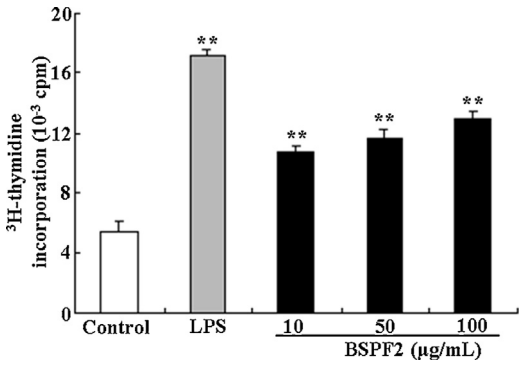

Fig. 4. Effects of BSPF2 on spleen cells proliferation. 5×10^5 mouse spleen cells were stimulated with 10, 50, 100 μg/mL of BSPF2 and positive inducer LPS at 5 μg/mL for 72 h. Cell proliferation was measured by ^{3}H-thymidine incorporation assay. Values are mean ± S.D. of triplicate determinations. LPS (5 μg/mL) serves as a positive control. Control is RPMI-1640. $^{**}p < 0.01$ compared with RPMI-1640 control.

by incorporation of ^{3}H-thymidine was determined in the treated spleen cells. As shown in Fig. 4, BSPF2 induced spleen cell proliferation in a dose-dependent manner. At 100 μg/mL, BSPF2 significantly enhanced spleen cell proliferation by about 2.4-fold. This maximum induction was slightly less than that of LPS at 5 μg/mL. Therefore, BSPF2 was characterized by a strong stimulation of proliferation of mouse spleen cells.

4. Conclusion

In the present study, a polysaccharide (BSPF2) was isolated from the tubers of *Bletilla striata*. BSPF2 was a novel galactomannan, with a main chain composed of (1 → 4)-linked mannosyl residues and (1 → 4)-linked glucosyl residues in a molar ratio of 2:1. The backbone was partially substituted at O-6 of glucosyl residues by galactosyl residues and mannosyl residues. Moreover, BSPF2 was found to stimulate spleen cells proliferation. On the basis of this finding, it was suggested that BSPF2 may be a good source for the development of immunomodulator in clinic. Further investigations are necessary to elucidate mechanisms responsible for its immunological activities.

Acknowledgements

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References

Alsop, R., & Vlachogiannis, G. (1982). Determination of the molecular weight of clinical dextran by gel permeation chromatography on TSK PW type columns. *Journal of Chromatography A*, *246*(2), 227–240.
Asamizu, T., & Nishi, A. (1979). Biosynthesis of cell-wall polysaccharides in cultured carrot cells. *Planta*, *146*(1), 49–54.
Bitter, T., & Muir, H. M. (1962). A modified uronic acid carbazole reaction. *Analytical Biochemistry*, *4*(4), 330–334.
Bradford, M. M. (1976). Rapid and sensitive method for quantitation of microgram quantities of protein utilizing principle of protein-dye binding. *Analytical Biochemistry*, *72*(1–2), 248–254.
Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A., & Smith, F. (1956). Colorimetric method for determination of sugars and related substances. *Analytical Chemistry*, *28*(3), 350–356.
De Belder, A., & Norrman, B. (1969). The substitution patterns of O-(2-hydroxyethyl) starch and O-(2-hydroxyethyl) dextran. *Carbohydrate Research*, *10*(3), 391–394.
Diao, H. J., Li, X., Chen, J. M., Luo, Y., Chen, X., Dong, L., et al. (2008). *Bletilla striata* polysaccharide stimulates inducible nitric oxide synthase and proinflammatory cytokine expression in macrophages. *Journal of Bioscience and Bioengineering*, *105*(2), 85–89.
Ghosh, K., Chandra, K., Roy, S. K., Mondal, S., Maiti, D., Das, D., et al. (2008). Structural investigation of a polysaccharide (Fr. I) isolated from the aqueous extract of an edible mushroom, Volvariella diplasia. *Carbohydrate Research*, *343*(6), 1071–1078.
Hu, C., Kong, Q., Yang, D., & Pan, Y. (2011). Isolation and structural characterization of a novel galactomannan from *Eremurus anisopterus* (Ker. et Kir) Regel roots. *Carbohydrate Polymers*, *84*(1), 402–406.
Lehrfeld, J. (1985). Simultaneous gas–liquid chromatographic determination of aldonic acids and aldoses. *Analytical Chemistry*, *57*(1), 346–348.
Liu, J. Y., Wang, H. C., Yin, Y., Li, N., Cai, P. L., & Yang, S. L. (2012). Controlled acetylation of water-soluble glucomannan from *Bletilla striata*. *Carbohydrate Polymers*, *89*(1), 158–162.
Navarini, L., Gilli, R., Gombac, V., Abatangelo, A., Bosco, M., & Toffanin, R. (1999). Polysaccharides from hot water extracts of roasted Coffea arabica beans: Isolation and characterization. *Carbohydrate Polymers*, *40*(1), 71–81.
Needs, P. W., & Selvendran, R. R. (1993). Avoiding oxidative-degradation during dodium-hydroxide methyl iodlde-mediated carbohydrate methylation in dimethyl-sulfoxide. *Carbohydrate Research*, *245*(1), 1–10.
Peng, Q., Lv, X., Xu, Q., Li, Y., Huang, L., & Du, Y. (2012). Isolation and structural characterization of the polysaccharide LRGP1 from *Lycium ruthenicum*. *Carbohydrate Polymers*, *90*(1), 95–101.
Rosenfeld, L., & Ballou, C. E. (1974). Acetolysis of disaccharides: Comparative kinetics and mechanism. *Carbohydrate Research*, *32*(2), 287–298.
Sweet, D. P., Shapiro, R. H., & Albersheim, P. (1975). Quantitative analysis by various g.l.c. response factor theories for partially methylated and partially ethylated alditol acetates. *Carbohydrate Research*, *40*(2), 217–225.
Tomoda, M., Nakatsuka, S., Tamai, M., & Nagata, M. (1973). Plant mucilages. VIII. Isolation and characterization of a mucous polysaccharide, Bletille-glucomannan, from *Bletilla striata* tubers. *Chemical & Pharmaceutical Bulletin*, *21*, 2667–2671.
Wang, C. M., Sun, J. T., Luo, Y., Xue, W. H., Diao, H. J., Dong, L., et al. (2006). A polysaccharide isolated from the medicinal herb *Bletilla striata* induces endothelial cells proliferation and vascular endothelial growth factor expression in vitro. *Biotechnology Letters*, *28*(8), 539–543.
Wu, Y. L., Sun, C. R., & Pan, Y. J. (2006). Studies on isolation and structural features of a polysaccharide from the mycelium of an Chinese edible fungus (*Cordyceps sinensis*). *Carbohydrate Polymers*, *63*(2), 251–256.