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# Structural characterization and *in vitro* fermentation of a novel polysaccharide from *Sargassum thunbergii* and its impact on gut microbiota

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ABSTRACT

The aim of the present study was to investigate structural characteristic and *in vitro* fermentation of a novel polysaccharide named ST-P2 from *Sargassum thunbergii* by human fecal inoculums, and its impact on human colonic microbiota. The results showed that ST-P2 was homogeneous with molecular weight of 48,788 Da, and consisted of arabinose, galactose, glucose, xylose, and mannose. The main linkage types were identified as (1 → 5)-α-L-Araf, (1 → 3)-α-L-Manp, (1 → 3,6)-β-D-Galp, (1 → 6)-α-D-Glcp, and (1 → 3)-β-D-Xylp, respectively. After 48 h fermentation, 67.83 ± 1.15% of total carbohydrate was utilized by colonic microbiota. The pH value in the fecal culture significantly decreased from 6.09 ± 0.11 to 4.70 ± 0.04. The concentrations of total short chain fatty acids, acetic, propionic, n-butyric and n-valeric acids significantly increased compared to the blank. ST-P2 could remarkably modulate the composition and abundance of beneficial microbiota. These results suggest that ST-P2 could potentially be a functional food aimed at promoting the gut health.

## 1. Introduction

Accumulating evidence has shown that various serious chronic diseases, such as type 2 diabetes, obesity, and colon cancer, are closely related to the disturbance of gut microbiota populations (Hand, Vujkovic-Cvijin, Ridaura, & Belkaid, 2016; Qin et al., 2012). In the last decades, considerable research has indicated that fermentation of dietary carbohydrate is beneficial for host and intestinal health by modulating colonic microbiota composition, lowering colonic pH, increasing production of short chain fatty acids (SCFAs), and stimulating colonic immune responses (Ma et al., 2017; Scott, Gratz, Sheridan, Flint, & Duncan, 2013). These carbohydrates can not be entirely digested or absorbed in the upper gastrointestinal tract and then reach large bowel where they are available for fermentation by colonic microbiota, thus conferring prebiotic effects (Gullón et al., 2014; Shang et al., 2016).

*Sargassum thunbergii*, belonging to the family of *Sargassum*, has been traditionally used as an edible and medicinal material in China and other Asian countries for centuries (Yuan, Zeng, Nie, Luo, & Wang, 2015). The polysaccharides from *S. thunbergii* have been found to exhibit various pharmacological effects such as antioxidant, hypoglycemic, immune, and antiproliferative activities (Kang et al., 2008; Luo et al., 2016; Ren et al., 2017). Our previous work has prepared a crude polysaccharide from *S. thunbergii* by hot water extraction. The crude polysaccharide was composed of arabinose, galactose, glucose, xylose, mannose, galacturonic acid, and glucuronic acid with molar percentages of 2.81, 23.2, 2.92, 20.8, 22.8, 9.74, and 17.7%, respectively. Moreover, the crude polysaccharide exhibited good antioxidant and hypoglycemic activities (Ren et al., 2017). However, to date, little information was available on the structure and fermentation of the polysaccharides from *S. thunbergii*.

Therefore, the aim of the present study was to investigate the structure and fermentation behavior *in vitro* of the polysaccharide from *S. thunbergii* by human fecal inoculums, and its impact on human gut microbiota. In order to precisely investigate the structural characteristics, a new polysaccharide named ST-P2 was prepared from *S. thunbergii* crude polysaccharide by purification with DEAE-Sepharose fast-flow column. The fermentation behaviors of ST-P2, including carbohydrate degradation, monosaccharide consumption, pH change, and SCFAs production in fecal cultures were investigated. Moreover, the fecal microbiota composition was analyzed using a MisSeq platform after sequencing of V3-V4 region of the 16 s rRNA gene.

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## 2. Materials and methods

### 2.1. Materials and chemical reagents

*S. thunbergii* was purchased from Changdao Market (Yantai, Shangdong, China). The dried sample was ground in a cutting mill and then passed through a 40-mesh sieve to obtain the powder (5.0% moisture). Acarbose, metformin, insulin, bovine serum albumin (BSA), trifluoroacetic acid (TFA), gastric lipase ($1.0 \times 10^5$ units/g), pepsin (3000 units/g), trypsin (300 units/mg), pancreatin (P745), and monosaccharide standards were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Bile salt was purchased from Beijing AOBOX Biotechnology Co., Ltd. (China). DEAE-Sepharose fast-flow was purchased from GE Healthcare Life Science (Uppsala, Sweden). Other chemicals and solvents were of analytical grade without further purification.

### 2.2. Preparation of polysaccharide

The crude polysaccharide from *S. thunbergii* was prepared according to the process described in our previous literature (Ren et al., 2017). The crude polysaccharide contained 30.8% of total carbohydrate, 2.22% of protein, and 11.4% of sulfate.

The crude polysaccharide (200 mg) was thoroughly dissolved with 5 mL deionized water, loaded onto the DEAE-Sepharose FF column (150 mL), and then kept quiet for 30 min. The column was sequentially eluted with 200 mL of distilled water and different concentrations of NaCl solutions (0.1, 0.2, 0.3, 0.4, 0.5, 0.6 M) at a flow velocity of 2 mL/min. Each tube with 5 mL of eluent was collected using an automatic collector (BSZ-100, Shanghai Precision Scientific Instrument Co., Ltd, China) and detected using the phenol-sulfuric acid method using glucose as the standard (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956). The elution curve was drawn using the tube number and absorbance (490 nm). Based on results of the elution curve (Fig. 1A), 0.3 M, 0.4 M, and 0.5 M NaCl eluents (named ST-P1, ST-P2, and ST-P3) were regarded as the main polysaccharide fractions. Before lyophilization, three eluents were collected, concentrated at 55 °C under reduced vacuum, and dialyzed against distilled water for 72 h at 4 °C with dialysis bags (3.0 kDa). ST-P2 accounting for 27.5% of crude polysaccharide was the highest among three fractions. Thus, the present study focused on ST-P2.

### 2.3. Chemical composition analysis

The contents of total carbohydrate, protein and sulfate of ST-P2 were measured by the phenol-sulfuric acid colorimetric method using glucose as the standard (Dubois et al., 1956), Commassie Brilliant Blue G-250 method using BSA as the standard (Bradford, 1976), and barium sulfate turbidimetry method (Ren et al., 2017), respectively.

### 2.4. Homogeneity and molecular weight (Mw) analysis

The homogeneity and Mw of ST-P2 were determined using high-performance gel permeation chromatography (HPGPC) (Ren et al., 2017). The process was performed on Agilent 1260 HPLC system (Santa Clara, CA, USA) including TSK-GEL guard column, G5000 $PW_{XL}$ column, and G3000 $PW_{XL}$ column in series coupled with an Agilent 1260 refractive index detector (RID). Briefly, ST-P2 solution (1 mg/mL) was prepared by ultrapure water and filtered through a 0.22 μm microporous filtering film before analysis. Thereafter, the filtrate (20 μL) was loaded onto the column, eluted with 0.02 M $KH_2PO_4$ (pH 6.0) at a flow velocity of 0.6 mL/min, and kept at 35 °C. The calibration curve was obtained using dextran standards.

### 2.5. Monosaccharide composition analysis

The monosaccharide composition of ST-P2 was determined by our previous method (Ren et al., 2017). Briefly, ST-P2 (5 mg) was dissolved in 4 mL of TFA in a sealed tube and kept at 105–110 °C for 6 h. Then, excess TFA was air-dried by a nitrogen gas blowing instrument. The residue was re-dissolved in 100 mL of deionized water and filtered through a 0.22 μm microporous filtering film before analysis. The filtrate was analyzed by a Dionex ICS 3000 system (Sunnyvale, CA, USA) equipped with a CarboPac PA1 guard column (50 × 2 mm) and a CarboPac PA20 analytic column (250 × 2 mm) coupled with pulsed amperometric detection (PAD). The column was eluted at a flow rate of 0.5 mL/min, and maintained at a temperature of 30 °C. The gradient elution was set as follows: 200 mM NaOH solution (0–2 min), 2 mM NaOH solution (3–20 min), and 200 mM NaOH solution (20–30 min). The injection volume was 10 μL.

### 2.6. Infrared (IR) spectrum analysis

ST-P2 (2 mg) was thoroughly mixed with 200 mg spectroscopic KBr powder, ground and then pressed into 1 mm pellet for analysis by a Vector 33 Fourier transform infrared spectrophotometer (Bruker, Ettlingen, Germany). The IR spectrum of ST-P2 was recorded in the wavenumber range of 500–4000 $cm^{-1}$ at a resolution of 2 $cm^{-1}$.

### 2.7. NMR spectroscopy analysis

ST-P2 (30 mg) was weighed and dissolved with 0.6 mL $D_2O$ in a NMR tube. The $^1H$ NMR and $^{13}C$ NMR spectra were recorded on a Bruker 600 MHz NMR apparatus (Bruker Corp., Fallanden, Switzerland) at 25 °C. All chemical shifts were expressed in ppm.

### 2.8. Triple-Helix structure analysis

The triple-helix conformation of ST-P2 was determined by the Congo-red method (Wang, Liu, & Qin, 2017) with some modifications. Briefly, ST-P2 (30 mg) was initially dissolved in 30 mL of deionized water and then mixed with 30 mL of Congo red solution (100 μM) with vigorous stirring. The maximum absorption wavelength ($\lambda_{max}$) values of the mixtures were measured at different concentrations of NaOH solutions (0–0.5 M). Congo red solutions without ST-P2 were used as the control.

### 2.9. Atomic force microscope (AFM) analysis

ST-P2 (5 mg) was dissolved in 5 mL of deionized water and kept stirring for 2 h at 60 °C. The ST-P2 solution was diluted to 2.5 μg/mL, kept stirring for another 12 h, and then filtered through a 0.22 μm microporous filtering film. Finally, 5 μL of filtrate was pipetted onto a fresh mica disk and dried in natural air. The mica disk was observed on the magnetic objective table of Tapping Mode AFM (Nanoscope 3A Multimode, Veeco Co., USA) at room temperature with a relative humidity of 65%. The image was captured with a silicon probe (Tap 150-G-10, Ted Pella, INC., USA) at a scanning frequency of 1 Hz and a scanning speed of 600 μm/s. Three-dimensional images were managed by WSxM software (Nanotec Electronica, Japan).

### 2.10. Preparation of human fecal inoculums

The fresh fecal inoculums were collected from three healthy donors who never had bowel disorders. Moreover, the donors consumed typical diet of the region preceding stool donation and did not get any treatment of antibiotics for at least three months. The fecal inoculums were mixed with an equal amount of feces from three donors in the sterile vials, diluted with 10% (v/v) Dulbecco's phosphate buffer to obtain a 20% (w/w) solution, and subsequently homogenized with a

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