A novel exopolysaccharide from *Weissella cibaria* FAFU821: Structural characterization and cryoprotective activity

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ABSTRACT

Exopolysaccharides produced by *Weissella cibaria* has attracted increasing attention owing to their biological activity. Here, a strain was isolated from the home-made fermented octopus, which was identified as *W. cibaria* FAFU821. In addition, the polysaccharide were isolated and purified by cellulose DE-52 column and Sephadex G-100 column, and named EPS821-1. In this work, the structure of EPS821-1 and its cryoprotective activity on *Bifidobacterium longum* subsp. *longum* F2 were investigated *in vitro*. These results suggested that the EPS821-1 is a novel glucan, which mainly consists of α-(1 → 6) linkage with α-(1 → 4), α-(1 → 4,6) and α-(1 → 3,6) residue as branches. In addition, EPS821-1 existed the three-dimensional network structure and exhibited the excellent cryoprotective activities for *B. longum* subsp. *longum* F2, which was 2.75 folds higher than that of the controls. This study provided scientific evidence and insights for the application of EPS821-1 as cryoprotection in food field.

1. Introduction

Microbial polysaccharide is an important and complex polymer, including exopolysaccharide, capsular polysaccharides and lipopoly-saccharides (Huang et al., 2022). Exopolysaccharide (EPS) as a secondary metabolite that can be secreted by bacteria (Pan et al., 2022). Recently, bacterial exopolysaccharide has received increasing attention from researchers owing to its variety of bioactivity, such as antimicrobial and anti-cancer activity (Feng et al., 2020; Li et al., 2022; Nehal, Sahnoun, Smaoui, Jaouadi, Bejar, & Mohammed, 2019; Rahbar Saadat, Yari Khosroushahi, & Pourghassem Gargari, 2019). It was reported that bacterial exopolysaccharide has been applied in the food industry owing to the unique physicochemical properties. Previously, Ayyash et al. found that exopolysaccharide from *Lactococcus garvieae* C47 possessed valuable health benefits and improved the structure of fermented camel milk (Ayyash et al., 2020). It has been reported that exopolysaccharide from lactic acid bacteria modified the rheological, texture and sensory properties of fermented products (Abedfar, Hosseininezhad, & Rafe, 2020; Hilbig, Gisder, Prechtl, Herrmann, Weiss, & Loeffler, 2019). Taken together, bacterial exopolysaccharide held a potential in the food industry.

Lactic acid bacteria are generally recognized as safe (GRAS), which have received extensive attention due to the qualified presumption of safety (QPS) status (Lobo et al., 2022; Mouafo, Sokamte, Mbawala, Ndjouenkeu, & Devappa, 2022). *Weissella* spp., belonging to the lactic acid bacteria family, was first discovered from fermented sausages in 1993 (Collins, Samelisl, Metaxopoulos, & Wallbanks, 1993). Remarkably, *W. cibaria* is one of the most well-known EPS producers (Yu, Jang, Lee, & Paik, 2019). According to the previous report, the application of exopolysaccharide from *W. cibaria* is closely associated with the structure. Annel *et al* has reported that *Weissel cibaira* BAL3C-5 and BAL3C-7 were high yield-producer dextran, which can be used as a food stabilizer in commercial bread (Hernandez-Alcantara et al., 2022). In addition, *W. cibaria* MED17 produced a dextran with (1 → 6)-linked α-D-glucose units, and it has the potential to be used as stabilizing and thickener agents for food industry (Aburas, Ispirli, Taylan, Yilmaz, & Dertli, 2020). Therefore, the biological activity and applications may be associated with the structures of EPS, including monosaccharide compositions and glycosidic linkages. To the best of our knowledge, this is the first report on the application of *W. cibaria* exopolysaccharide to cryoprotective application of *Bifidobacterium*.

In this work, *W. cibaria* FAFU821 was isolated from home-made

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fermented octopus. Exopolysaccharide from *W. cibaria* FAFU821 (EPS821) was extracted, isolated and purified with cellulose DE-52 column and Sephadex G-100 column. Finally, one purified fraction with the highest content (named EPS821-1) was obtained from *W. cibaria* FAFU821. The structure of EPS821-1, including monosaccharide compositions, molecular weight, functional group, glycosidic bonds and surface microstructure were investigated by HPLC, FT-IR, GC-MS, NMR and SEM. Finally, EPS821-1 was proven to have cryoprotective effects on *B. longum* subsp. *longum* F2, which would provide a new insight into its application in food field.

2. Material and methods

2.1. Microorganisms

W. cibaria FAFU821 was isolated from the home-made fermented octopus using the method described previously with modifications (Yilmaz, Ispirli, Taylan, Alamoudi, & Dertli, 2022). The home-made fermented octopus was cut into small pieces, the sample was diluted with PBS and inoculated on MRS medium supplemented with 100 g/L sucrose at 37 °C for 24 h. Here, a slimy colony was inoculated on MRS medium. The genomic DNA was identified using Sanger sequencing, and the morphology of *W. cibaria* FAFU821 was detected by gram staining and scanning electron microscope.

2.2. Extraction and purification of EPS821

Extraction, isolation and purification of exopolysaccharide from *Weissella cibaria* FAFU821 (EPS821) was performed according to the previous method with minor modifications (Hu et al., 2020). Briefly, *W. cibaria* FAFU821 (2 %, w/v) was added in MRS medium with 100 g/L sucrose at 30 °C for 36 h. EPS821 was precipitated using the three-fold volume of cold 95 % (v/v) ethanol and then incubated overnight at 4 °C. Then, the mixture was centrifuged at 4000 rpm (cence, L550, Changsha, China) for 10 min to collect the precipitation. The precipitation was re-dissolved in deionized water and the protein of EPS821 was removed by a concentration 5 % (w/v) of trichloroacetic acid at 4 °C for 8 h. Three-fold volume of cold 95 % (v/v) ethanol were added to the supernatant followed by incubation at 4 °C. The precipitates were centrifugation at 4000 rpm for 10 min, the crude EPS821 was dialyzed with deionized water at 4 °C for 48 h.

The crude EPS821 was isolated by cellulose DE-52 column (2.6 cm × 30 cm) and eluted with deionized water, 0.1 M and 0.3 M NaCl at a flow rate of 2.0 mL/min, respectively. The EPS821 fraction with the highest content (EPS821-1) was purified by a Sephadex G-100 gel column (1.5 cm × 50 cm) with deionized water (flow rate = 0.5 mL/min). The polysaccharide content of eluent was measured by the phenol–sulfuric acid method (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956). The EPS821-1 was lyophilized for subsequent experiments.

2.3. UV visible spectrum analysis

The EPS821-1 was re-dissolved in deionized water and prepared at a concentration of 0.5 mg/mL. UV–Vis absorption spectra were recorded by the UV–Vis Spectrophotometer (Thermo Scientific, Wilmington, USA) in a wavelength range of 190–400 nm to examine the existence of nucleic acid and protein.

2.4. Monosaccharide composition

The HPLC was used to determined monosaccharide composition according to our described method (Pan, Wang, Zhang, Zhang, & Zheng, 2023). Briefly, 4 mg EPS821-1 was hydrolyzed by trifluoroacetic acid (2 mL, 2 M) at 110 °C for 4 h, dried under N_2 and washed with chromatographic methanol to remove TFA. The residues were dissolved using 500 μL deionized water. EPS821-1 was derivatized by NaOH (100

μL, 0.3 M), and PMP (100 μL, 0.5 M) was supplemented to incubate at 70 °C for 1 h. Next, HCl (100 μL,0.3 M) and 1 mL chloroform was supplemented to the samples. Lastly, the 0.22 μm membrane was using to filter samples. Monosaccharide compositions of EPS821-1 was analyzed by HPLC (Waters Inc., USA). The system was equipped with Waters SunFire C18 column (4.6 × 250 mm). This mobile phase was phosphate buffer and acetonitrile (83: 17, v/v,) at 25 °C (flow rate = 0.5 mL/min, wavelength range: 245 nm).

2.5. Molecular weight

The molecular weight (M_w) of EPS821-1 was analyzed by HPGPC. Sample was mixed with 0.05 M NaCl to 5 mg/mL concentration solution, following centrifuging at 12,000 rpm for 10 min. The 0.22 μm membrane was using to filter supernatant. The HPGPC was equipped with a RID-10A detector. The mobile phase was 0.05 M NaCl (flow rate = 0.6 mL/min), and 25 μL sample was injected.

2.6. FT-IR spectrum

FT-IR spectra of EPS821-1 was recorded by FT-IR system (Nicolet iS20, Thermo Scientific, USA). In short, 2 mg EPS821-1 was dried and mixed with KBr and pressed into flakes (the spectrum wavelength: 4000–400 cm^{-1}).

2.7. NMR analysis

The EPS821-1 was dissolved in deuterium oxide. ^{1}H NMR, ^{13}C NMR, HSQC, COSY and HMBC spectra of the EPS821-1 was obtained on a Bruker 400 MHz spectrometer (Bruker, USA) at 300 K (26.85 °C).

2.8. Methylation analysis

Glycosidic bonds of EPS821-1 was identified by methylation analysis according to reported methods (Zhang et al., 2021). In brief, the polysaccharide was dissolved in DMSO at a concentration of 4 mg/mL. The solution was methylated in DMSO/NaOH with CH$_3$I. Then, the per-methylated products were hydrolyzed with 2 M TFA at 121 °C for 1.5 h, reduced by NaBD$_4$ and acetylated with acetic anhydride at 100 °C for 2.5 h. 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. Mass spectrometry analysis was performed at the initial temperature of 140 °C for 2.0 min, and the temperature was increased to 230 °C by 3 °C/min for 3 min. The scan mode was SCAN with a range from 30 to 600 *m/z*.

2.9. Scanning electron microscopy

Surface structure and morphology of EPS821-1 was determined by scanning electron microscopy (TM3030Plus, HITACHI, Japan). Briefly, fixed freeze-dried EPS821-1 on a copper platform and samples were sputtered with gold powder for 2 min. Images were recorded at 500×, 1000×, 2500× magnification.

2.10. Cryoprotective activity

The cryoprotective effect of EPS821-1 on *B. longum* subsp. *longum* F2 at -80 °C were investigated according to the previous methods with minor modifications (Zhang, Che, & Wu, 2022). Briefly, *B. longum* subsp. *longum* F2 was cultured to logarithmic phase in the BAKER RUSKINN anaerobic workstation (Concept 400, Ruskinn Technology, Ltd., UK) and mixed with equal volumes of 0.9 % saline, 20 % glycerol or 3 % (w/v) EPS821-1 solutions. Samples were frozen at -80 °C for 7 days. Plating colony-counting method was used to calculate the survival rate of

B. longum subsp. *longum* F2.

2.11. Statistical analysis

The results were performed in triplicate, and the data was presented as mean ± SEM. Statistical analysis was performed using SPSS 23.0 software. $p < 0.05$ was indicated a significant difference.

3. Results and discussion

3.1. Isolation and purification of EPS821-1

The strain FAFU821 was identified by morphological characteristics and 16S rDNA sequencing, the results showed that FAFU821 belonged to *Weissella cibaria*, rod-shaped, gram-positive (Fig. 1a-1b). The yield of crude exopolysaccharide from *Weissella cibaria* FAFU821 (EPS821) was 4.88 ± 0.75 g/L. Moreover, the crude EPS821 was separated by the cellulose DE-52 column, and three EPS peaks were observed in Fig. 1c. Lastly, the highest content polysaccharide fraction EPS821-1 was eluted by Sephadex G-100 column. As showed in Fig. 1d, one major EPS peak was obtained in EPS821-1. The lack of signals at 260 nm and 280 nm indicated the absence of nucleic acid and protein (Fig. 1e).

3.2. Monosaccharide composition of EPS821-1

The monosaccharide composition of EPS821-1 was showed in Fig. 2a. The analysis revealed that EPS821-1 was mainly consisted of glucose, accounting for 98.63 % of the total content. In addition, a small amount of mannose was presented, and the proportion was 0.55 %. These findings indicated that EPS821-1 belong to glucan. It has been reported that *W. cibaria* CMGDEX3, *W. cibaria* MED17 and *W. cibaria* RBA12 are common microorganisms that synthesized glucan (Aburas et al., 2020; Ahmed, Siddiqui, Arman, & Ahmed, 2012; Baruah, Maina, Katina, Juvonen, & Goyal, 2017). Interestingly, exopolysaccharide from *W. cibaria* SJ14 contained varies monosaccharide in differences ratios, while *W. cibaria* MD2 exopolysaccharide only included fructan (Table 1) (Lakra, Ramatchandirane, Kumar, Suchiang, & Arul, 2021; Zhu et al.,

2018). Therefore, we speculate that the monosaccharide composition of exopolysaccharide may be related to the strain and the components of the medium.

3.3. Molecular weight of EPS821-1

The molecular weight (M_w) of EPS821-1 was analysed by HPGPC. As shown in Fig. 2b, the M_w of EPS821-1 was estimated to be 1373058 Da. It has been reported that the molecular weight of exopolysaccharide from *Weissella cibaria* NC516.11 is 2.82×10^6 Da (Li, Ai, Xu, Hu, Yao, & Wang, 2022). In addition, Yu *et al* found that the molecular weight of exopolysaccharide produced by *Weissella cibaria* W27 is 1.9×10^7 Da in MRS medium adding 20 g/L sucrose, and the molecular weight improved to 3.9×10^7 Da with MRS medium containing 60 g/L sucrose (Yu, Chen, Chen, & Ng, 2018). Generally, the molecular weight of exopolysaccharide is positively correlated with sucrose content of the medium (Son et al., 2007). In summary, the molecular weight of exopolysaccharide related to bacterial strain, culture conditions and culture medium compositions.

3.4. FT-IR analysis of EPS821-1

The functional groups of EPS821-1 was performed by FT-IR analysis as showed in Fig. 2c, the signal at 3404 cm^{-1} was displayed by hydroxyl groups (Li et al., 2022). Three signals were observed at 2929, 1420 and 1272 cm^{-1} owing to the C–H stretch vibration (He, Ye, Du, Wang, Wu, & Yang, 2014; Wu, An, Liu, Hu, Wang, & Zou, 2022; Zhang, Che, et al., 2022). Here, the signal around 1649 cm^{-1} was corresponded to bound water (Zhu et al., 2018). The signal at 916 cm^{-1} was induced by pyranose of the glycosyl residues (Yalmanci, İspirli, & Dertli, 2022), while a weak band was caused around 1156 cm^{-1}, indicating C–O–C stretching vibration, which was carbohydrate characteristic signal (Tian, Zhao, Zeng, Zhang, & Zheng, 2016). Moreover, a wide peak at the 1015 cm^{-1} was observed, which revealed the presence of the $(1 \rightarrow 6)$-linked α-D-glucose (Aburas et al., 2020). In particular, the signal at 885 cm^{-1} was lack, demonstrating the non-existent of β-configuration (He et al., 2014). Therefore, the FT-IR analysis proved that EPS821-1 contained $(1 \rightarrow 6)$
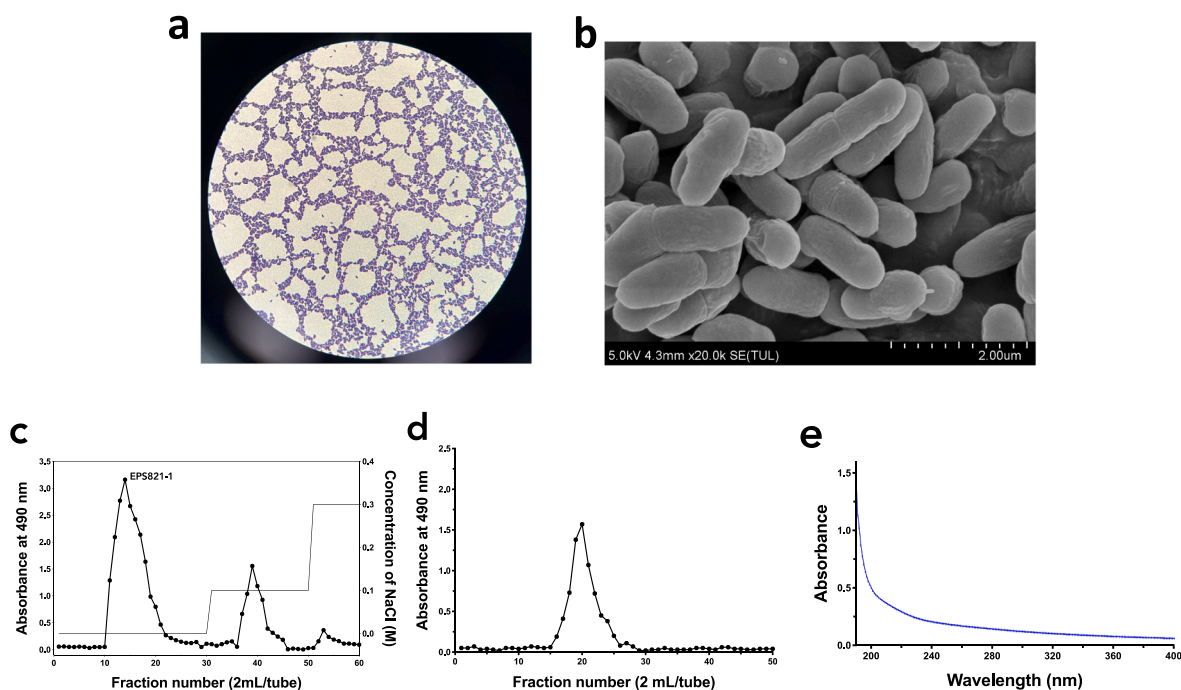

Fig. 1. (a-b): Morphological characteristics of the strain FAFU821; (c-d) Isolation and purification of EPS. (c): cellulose DE-52 column, (d): Sephadex G-100 column; (e): The UV spectrum of EPS821-1.
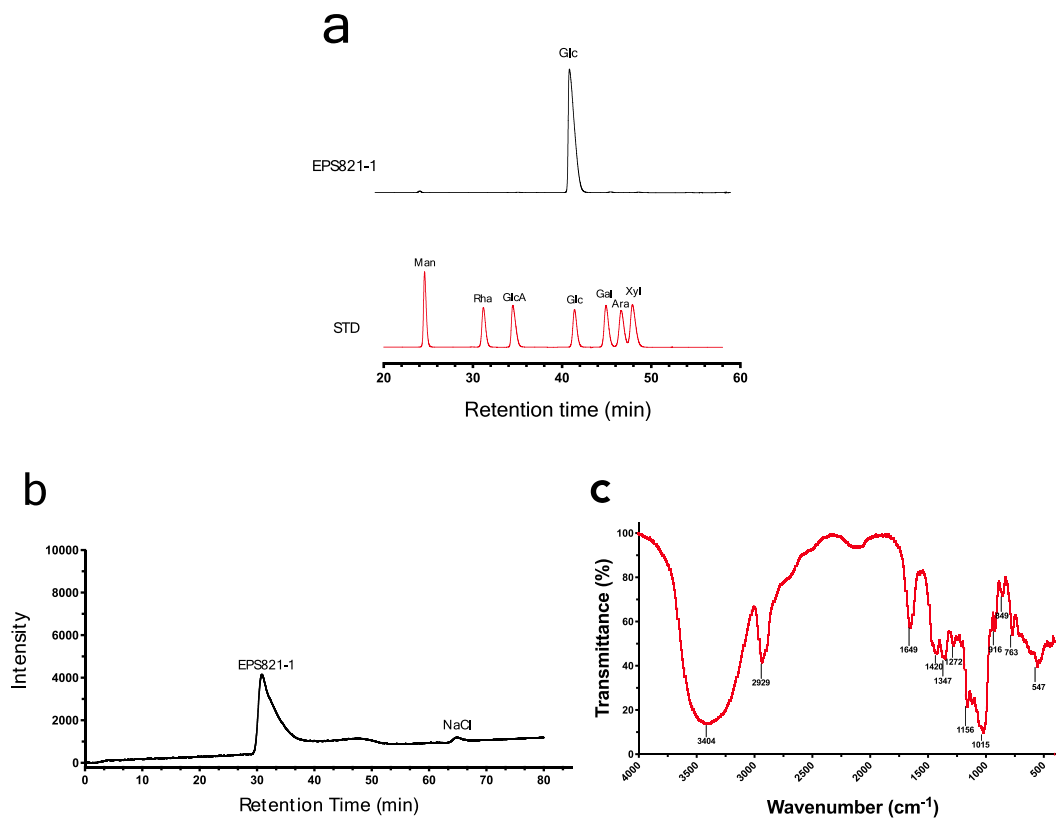
Fig. 2. (a) Monosaccharide compositions of EPS821-1; (b): Molecular weight of EPS821-1; (c): Fourier-transform infrared (FTIR) spectra of EPS821-1.

Table 1
Monosaccharide composition of exopolysaccharide from *W. cibaria*.

| Strain	Monosaccharide Composition
W. cibaria CMGDEX3	Glucan
W. cibaria MED17	Glucan
W. cibaria RBA12	Glucan
W. cibaria SJ14	EPS-1_Mannose: Glucose: Galactose: Arabinose: Xylose: Rhamnose = 23.79: 4.80: 1.66: 1.00: 0.21: 0.09 EPS-2_Galactose: Mannose: Glucose: Arabinose = 7.47: 3.69: 1.00: 0.85
W. cibaria MD2	Fructan

linked.

3.5. NMR spectroscopy analysis of EPS821-1

1D NMR and 2D NMR were employed to analyze the structure of EPS821-1, which results were shown in Fig. 3a-3e. In the ^{1}H NMR spectra, the majority of signals divided into two parts: anomeric region and ring proton region, which the signal distribution were 4.5-5.5 ppm and 3.1-4.5 ppm. Anomeric region was assigned to H-1 protons, and proton region attributed to the protons assigned to H2-H6 (Zhang, Zeng, et al., 2022). The ^{1}H NMR spectrum of EPS821-1 presented only two signals in the anomeric region (Fig. 3a), the intense signal peak at 4.86 ppm and the low intensity anomeric peak at 5.21 ppm, which associated to α-(1 → 6) linkages and α-(1 → 3) linkages, respectively (Bisson, Comuzzi, Giordani, Poletti, Boaro, & Marino, 2023). The ^{13}C NMR spectrum of EPS821-1 contained anomeric carbons regions and ring carbons regions, which the signal distribution were 95-110 ppm and 50-85 ppm (Fig. 3b). The anomeric carbon peak at 97.65 ppm demonstrated that EPS821-1 contained α-configuration (Zhang et al., 2021). Owing to the limitations of 1D NMR in structural characterization of EPS821-1, 2D

NMR was employed to further analyze the EPS821-1 structure. The directly connected C—H relationship was shown in Fig. 3c, HSQC spectrum of EPS821-1 with signals 4.86/97.65, 3.48/71.34, 3.61/73.35, 3.41/69.46, 3.81/70.12 and 3.89, 3.66/65.47 attributed to H1/C1, H2/*C*2, H3/C3, H4/C4, H5/C5 and H6, H6′/C6, respectively. In addition, the COSY spectrum presented the relationship between H on adjacent C (Fig. 3d). Owing to the signal peak of α-(1 → 3) linkages was weak, it cannot be clearly displayed in HSQC spectrum, while it can be clearly seen in ^{1}H NMR spectrum. As shown in Fig. 3e, the long-range correlations of EPS821-1 between C6 and H1 verified the existence of α-(1 → 6) linkage in the HMBC spectrum (Wang, Zhang, Wang, & Pan, 2023). Moreover, HMBC spectrum showed that EPS821-1 presented α-(1 → 4) linkage and α-(1 → 3) linkage. Similarly, the NMR spectra of *W. cibaria* PDER21 exopolysaccharide showed that the glucose residues were observed at 4.88 ppm and a strong signal at 97.6 ppm, indicating PDER21 composed of (1 → 6) linkage and (1 → 3) linkage (Yilmaz et al., 2022). Likewise, an exopolysaccharide from *W. cibaria* RBA12 observed to be a dextran with α-(1 → 6) linkage as main chain and α-(1 → 3) linkage as branches in NMR spectra (Baruah et al., 2017). Aburas *et al* found that *W. cibaria* MED17 exopolysaccharide existed α-(1 → 6) linkage as main chain and α-(1 → 3) linkage as branches from 1D NMR spectra (Aburas et al., 2020).

3.6. Glycosidic bond analysis of EPS821-1

Methylation analysis was conducted to determined the linkage type of EPS821-1 by GC–MS. As shown in Table 2, one peak observed at 1,5-di-O-acetyl-2,3,4,6-tetra-O-methyl glucitol in EPS821-1, verifing EPS821-1 was non-reducing glucopyranose. Moreover, EPS821-1 was composed of Glcp-(1→, →6)-Glcp-(1→, →3,6)-Glcp-(1→, →4)-Glcp-(1→, →4,6)-Glcp-(1 → glycosidic bond with the molar percentage of 8.179 %, 82.754 %, 1.294 %, 5.662 % and 2.112 %, respectively. These results proved that the major linking type of EPS821-1 was (1 → 6) glycosidic bond, which was consistent with the results of
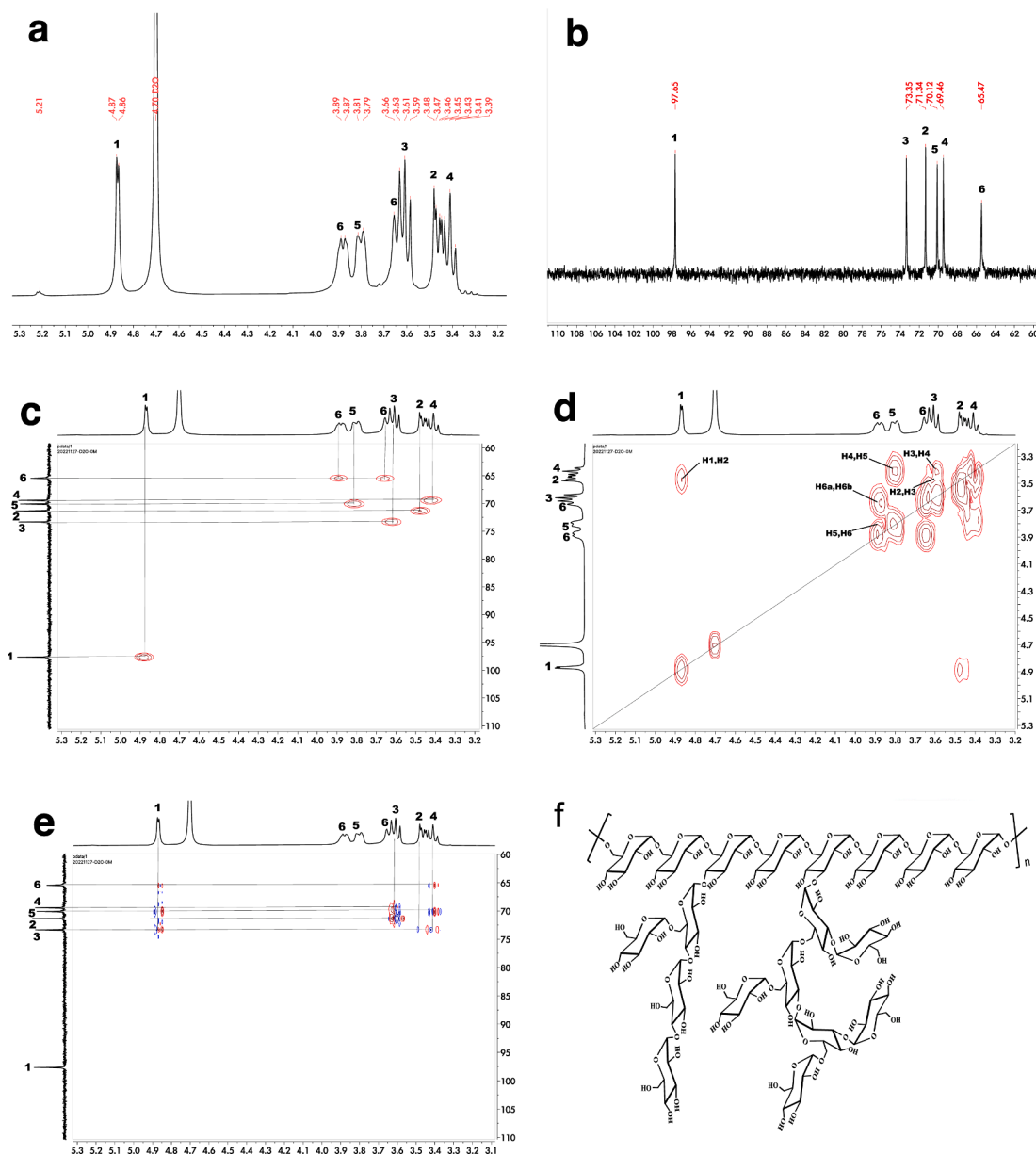
Fig. 3. (a–e): The 1D NMR spectra of EPS821-1 and 2D NMR spectra, (a): ^{1}H NMR spectra, (b): ^{13}C NMR spectra, (c): HSQC, (d): COSY and (e): HMBC; (f): Preliminary structure of EPS821-1.

Table 2
Methylation analysis of EPS821-1.

Retention Time (min)	Methylated Sugars	Linkage types	Relative Molar Ratios (%)
8.287	1,5-di-O-acetyl-2,3,4,6-tetra-O-methyl glucitol	Glc*p*-(1→	8.179
13.017	1,5,6-tri-O-acetyl-2,3,4-tri-O-methyl glucitol	→6)-Glc*p*-(1→	82.754
13.283	1,4,5-tri-O-acetyl-2,3,6-tri-O-methyl glucitol	→4)-Glc*p*-(1→	1.294
16.9	1,3,5,6-tetra-O-acetyl-2,4-di-O-methyl glucitol	→3,6)-Glc*p*-(1→	5.662
17.467	1,4,5,6-tetra-O-acetyl-2,3-di-O-methyl glucitol	→4,6)-Glc*p*-(1→	2.112

monosaccharide analysis. NMR and methylation analysis determined that EPS821-1 was consisted of α-(1 → 6) linkage with α-(1 → 4), α-(1 → 4,6) and α-(1 → 3,6) brances. Therefore, these dates indicated that

EPS821-1 might be a glucan with different ratio and sugar residues. Smilarly, Zhang *et al* found that EPS produced by *Russula vinosa Lindblad* (WRP2 and WRP3) might be the same type of galactoglucan and the degrees of branching of WRP2 and WRP3 decreased during the fractionation process (Zhang et al., 2021). Unfortunately, owing to the low content, the mannose could not be detected by methylation. Likewise, Gan *et al* found an EPS produced by *Halomonas saliphila* LCB16^T mainly consisted of mannose and due to the low amounts of several monosaccharides, only mannose and glucose derivatives were detected in methylation analysis (Gan et al., 2020). There is ample indication that EPS821-1 was a glucan, including α-(1 → 6) linkage as the core structure with α-(1 → 4), α-(1 → 4,6) and α-(1 → 3,6) brances (Fig. 3f).

3.7. Microstructure of EPS821-1

The microstructure of EPS821-1 was observed by SEM. The EPS821-1 revealed a highly arranged three-dimensional structure, which is similar to a porous network (Fig. 4a). In addition, more details of the
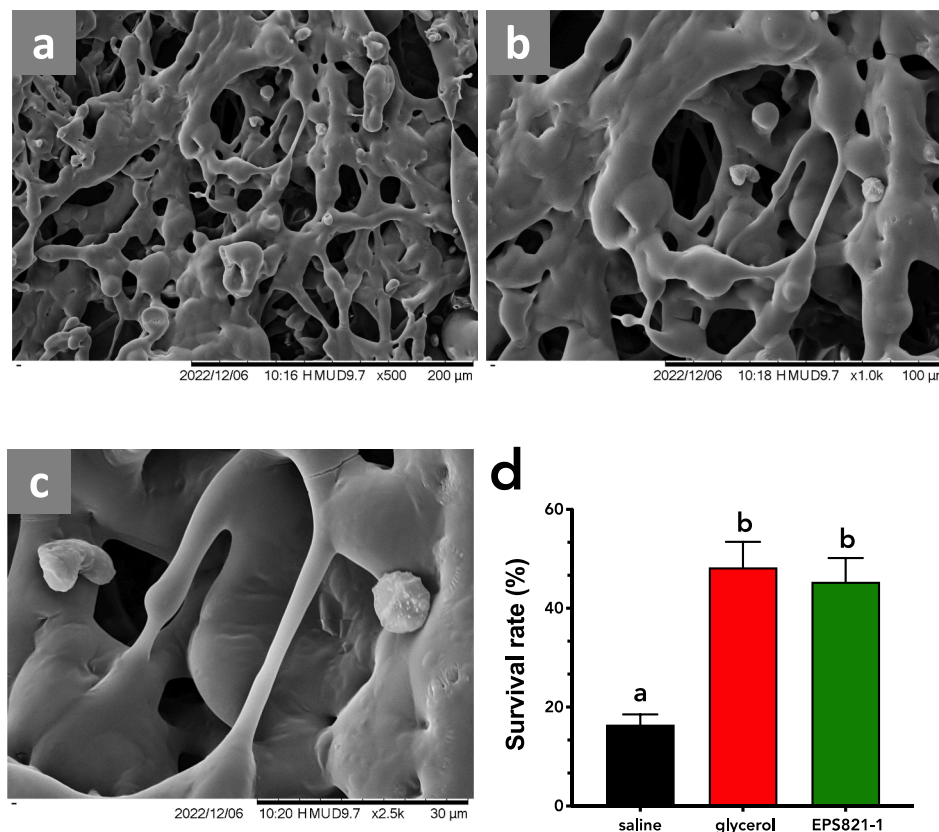
Fig. 4. (a-c): Scanning electron microscopy of EPS821-1 (a-c, magnification 500×, 1.0 k× and 2.5 k×); (d): Cryoprotective activity of EPS821-1 on *B. longum* subsp. *longum* F2.

microstructure of EPS821-1 were characterized at a higher magnification (Fig. 4b-4c). It is reported that polysaccharides presented rough and hollow structure, which is conducive to the entry of water molecules and stable structure formation. Previously, Wang *et al* found that exopolysaccharide produced by *L. plantarum* YW11 had a similar three-dimensional structure and smooth surface (Wang, Zhao, Tian, Yang, & Yang, 2015). Saravanan *et al* reported that exopolysaccharide from *Leuconostoc lactis* KC117496 showed compact and porous structure, which was essential for the water holding capacity (Saravanan & Shetty, 2016). Therefore, we speculate that EPS821-1 could improve the water holding capacity of food due to the unique three-dimensional network and porous structure.

3.8. Cryoprotective activities of EPS821-1

The cryoprotective effect of EPS821-1 on *B. longum* subsp. *longum* F2 was determined with 20 % glycerol as positive control (Fig. 4d). After frozen for 7 days, *B. longum* subsp. *longum* F2 with 20 % glycerol showed the highest survival rate (48.25 %). Here, *B. longum* subsp. *longum* F2 was protected by 0.9 % saline and 3 % (w/v) EPS821-1 with the survival rates of 16.47 %, 45.37 %, respectively. Overall, *B. longum* subsp. *longum* F2 exhibited better viability towards cold stress in the present of 3 % EPS821-1 and 3 % glycerol compared to 0.9 % saline. It has been reported that a polysaccharide from Antarctic *Pseudomonas* sp. ID1 was mainly composed of glucose, galactose and fucose with a molecular weight higher than 2×10^6 Da, which has the cryoprotective activity to *E. coli* ATCC 10536 (Carrion, Delgado, & Mercade, 2015). In addition, the polysaccharide from *Mucilaginibacter* sp. ERMR7:07 mainly contained $(1 \rightarrow 6)$ glycosidic linkage, which provided better cryoprotection for *E. coli* MTCC 43 (Kumar, Mukhia, & Kumar, 2022). Moreover, Zhang *et al.* reported an EPS from *Zygosaccharomyces rouxii* was composed of galactose, glucose and mannose and exhibited the porous structure,

which was cryoprotective against *L. lactis* MG 1363 (Zhang, Che, et al., 2022). In particular, EPS produced by *Tetragenococcus halophilus*, which has a component EPS-1 with a three-dimensional structure, provided better cryoprotection than EPS-2 with an irregular lamellar structure (Zhang, Zeng, et al., 2022). Taken together, there are examples indicating that the cryoprotective activity of polysaccharides is related to the indicator bacteria and the structure of polysaccharides, including molecular weight, monosaccharide composition, glycosidic bonds, and surface structure. Therefore, we hypothesize that EPS821-1 could be further explored as cryoprotectant in microorganism.

4. Conclusion

Weissella cibaria FAFU821 was obtained from the home-made fermented octopus, and exopolysaccharide from *W. cibaria* FAFU821 was isolated and purified in this work. Results suggested that the highest content of polysaccharide fraction EPS821-1 was glucan by α-$(1 \rightarrow 6)$ glycosidic bond as the main chain with the branches of α-$(1 \rightarrow 4)$, α-$(1 \rightarrow 4,6)$ and α-$(1 \rightarrow 3,6)$. In particular, EPS821-1 exhibited excellent cryoprotective role on *B. longum* subsp. *longum* F2, while the application of purified EPS821-1 and crude EPS821 in food needs further investigation.

CRediT authorship contribution statement

Fan Zhang: Investigation, Data curation, Formal analysis, Writing – original draft. **Lin Wang:** Investigation, Writing – review & editing. **Zihao Zhang:** Investigation, Writing – review & editing. **Baodong Zheng:** Conceptualization, Project administration. **Yi Zhang:** Conceptualization, Project administration. **Lei Pan:** Writing – original draft, Project administration, Funding acquisition.

Declaration of Competing Interest

Data availability

Data will be made available on request.

Acknowledgements

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