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Preparation and structural elucidation of a glucomannogalactan from marine fungus *Penicillium commune*

Yanli Chen, Wenjun Mao*, Junfeng Wang, Weiming Zhu, Chunqi Zhao, Na Li, Chunyan Wang, Mengxia Yan, Tao Guo, Xue Liu

Key Laboratory of Marine Drugs, Ministry of Education, School of Medicine and Pharmacy, Ocean University of China, 5 Yushan Road, Qingdao 266003, People's Republic of China

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ABSTRACT

The coral-associated fungus *Penicillium commune* produces an extracellular polysaccharide, FP2-1, when grown in potato dextrose-agar medium. FP2-1 was isolated from the fermented broth using anion-exchange and size-exclusion chromatography, and its structure was elucidated by chemical and spectroscopic analyses, including detailed nuclear magnetic resonance spectroscopy. The results showed that FP2-1 was a glucomannogalactan with a molar ratio of galactose, mannose and glucose of 3.9:1.9:1.0. Structure of FP2-1 may be represented, at an average, as a backbone of (1→2)-linked α-mannopyranose with the every second residue substituted at position 6 by a pentasaccharide branch. The branches consist of four (1→6)-linked β-galactofuranose residues with terminal α-glucopyranose residue attached to the last galactofuranose residue at position 2. FP2-1 was a novel galactofuranose-containing extracellular polysaccharide differing from previously described extracellular polysaccharides.

1. Introduction

Symbiotic and epiphytic microorganisms of large marine organisms have close relationship with the hosts (Croft et al., 2005). Recently, some investigations indicated that symbiotic and epiphytic microorganisms may be involved in organism biosynthesis, metabolism and other life activities of large hosts (Dobretsov & Qian, 2004; Osinga et al., 2001). Interest in extracellular polysaccharides produced by symbiotic and epiphytic microorganisms is increasing, and the extracellular polysaccharides could be explored as a source of useful polymers (Chen et al., 2012).

Polysaccharides with hexofuranose units are attractive due to their unique structures and specific properties (Nakajima & Ichishima, 1994). Galactose is by far the most widespread hexose in furanose form in naturally-occurring polysaccharides (Peltier, Euzen, Daniellou, Nugier-Chauvin, & Ferrières, 2008). Galactofuranose-containing polysaccharides presenting in some fungi show chemical characteristics different from those of other extracellular polysaccharides (Gómez-Miranda et al., 2003; Shibata & Okawa, 2011). However, the structures of galactofuranose-containing polysaccharides produced by symbiotic and epiphytic microorganisms have not yet been fully characterized. In the present work, a galactofuranose-containing extracellular polysaccharide was obtained from the fermented broth of the marine fungus *Penicillium commune* from the coral *Muricella abnormalis*, and its structure was investigated by chemical and spectroscopic methods, including one- and two-dimensional nuclear magnetic resonance (1D and 2D NMR) spectroscopic analyses.

2. Materials and methods

2.1. Materials

Q Sepharose Fast Flow and Sephacryl S-100 were from GE healthcare (Piscataway, NJ, USA). Dialysis membranes (flat width 44 mm, molecular weight cut off of 3500) were from Lvniao (Yantai, China). Monosaccharide standards (L-arabinose, L-fucose, D-xylose, D-mannose, D-galactose, D-glucose, D-glucuronic acid, D-galacturonic acid and D-glucosamine) were purchased from Sigma–Aldrich (St. Louis, MO, USA). Dextran standards (M_w: 200, 107, 47.1, 21.1, 9.6 and 5.9 kDa) were from Showa Denko K.K. (Tokyo, Japan).

2.2. Strains and fermentation

P. commune was isolated from coral *M. abnormalis* collected from Danzhou, Hainan province of China. The producing strain was prepared on Potato Dextrose agar slants at 3.33% salt concentration and stored at 4 °C. *P. commune* was grown under static conditions

* Corresponding author. Tel.: +86 532 8203 1560; fax: +86 532 8203 3054.
E-mail address: wenjunmqd@hotmail.com (W. Mao).

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at 28 °C for 10 days in the liquid medium. The liquid medium was composed of mannitol (2.0%), maltose (2.0%), monosodium glutamate (1.0%), KH_2PO_4 (0.05%), corn syrup (0.1%), glucose (1.0%), $MgSO_4{\cdot}7H_2O$ (0.03%), $CaCO_3$ (2.0%), yeast extract (0.3%) and sea salt (3.3%), pH 6.5. The fermented whole broth of about 40 L was obtained.

2.3. Preparation of the extracellular polysaccharide

The fermented broth was filtered through cheesecloth to separate into filtrate and mycelia. The filtrate was concentrated to 1/4 of its original volume under reduced pressure at 40 °C, and extracted three times by adding same volume of ethyl acetate (1.0 h) to remove the lipophilic compounds. The aqueous layer was concentrated under reduced pressure at 40 °C and a three-fold of the volume of 95% (v/v) ethanol was added. The resulting precipitate was recovered by centrifugation at 8000 rpm for 10 min, dialyzed in cellulose membrane tubing against flowing distilled water for 48 h. The retained fraction was vacuum-dried, and the protein in the fraction was removed (Matthaei, Jone, Martin, & Nirenberg, 1962). The crude polysaccharide was fractionated by a Q Sepharose Fast Flow column (50 cm × 2.6 cm) coupled to an AKTA FPLC system, eluted with a step-wise gradient of 0, 0.08 and 0.2 mol/L NaCl. The fractions were assayed for carbohydrate content (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956). Fractions eluted with 0.08 mol/L NaCl were pooled, dialyzed, loaded onto a Sephacryl S-100 column (70 cm × 2.6 cm), and eluted with 0.2 mol/L NH4HCO3 at a flow rate of 0.3 mL/min. The major polysaccharide fractions were pooled and freeze-dried. After these steps, a purified polysaccharide was obtained, and designated as FP2-1.

2.4. General analysis

The homogeneity and molecular weight were measured by high performance gel permeation chromatography (HPGPC) on a Shodex OHpak SB-804 HQ column (7.8 mm × 300 mm, Tokyo, Japan), and the molecular weight was estimated by reference to a calibration curve made by dextran standards (Sun et al., 2009). Total sugar content was determined by the phenol-sulfuric acid method using galactose as the standard (Dubois et al., 1956). Uronic acid content was measured by the carbazole-sulfuric acid method using glucuronic acid as standard (Bitter & Muir, 1962). Sulfate content was assessed according to Silvestri, Hurst, Simpson, and Settine (1982). Protein content was assayed as described by Bensadoun and Weinstein (1976).

2.5. Partial acid hydrolysis

Partial acid hydrolysis of FP2-1 was performed according to Ishurd and Kennedy (2005). The polysaccharide was degraded by 0.01 mol/L trifluoroacetic acid at 105 °C for 1.5 h. The hydrolysate was evaporated to dryness, and methanol was added and evaporated to dryness again until no acidic vapor was present. The dry sample was dissolved in 50% ethanol. The soluble and insoluble products were recovered by centrifugation (8000 rpm, 10 min), vacuum-dried and designated as FP2-1S and FP2-1P, respectively.

2.6. Analysis of monosaccharide composition

Monosaccharide composition was determined by reversed-phase high performance liquid chromatography (HPLC) after pre-column derivatization and UV detection. Briefly, polysaccharide was hydrolyzed with 2 mol/L trifluoroacetic acid at 100 °C for 6 h in a sealed tube. Excess acid was removed by co-distillation with methanol for four times after the hydrolysis was completed. 1 mg of dry hydrolysate was dissolved in 100 μL of 0.3 mol/L NaOH, and then added to 120 μL of 0.5 mol/L methanol solution of 1-phenyl-3-methyl-5-pyrazolone (PMP) at 70 °C for 1 h. Finally, the mixture was added 100 μL of 0.3 mol/L HC1 solution and vigorously shaken and centrifuged for 5 min. The supernatant was filtered through 0.22 μm nylon membranes (Westborough, MA, USA) and 10 μL of the resulting solution was injected into the XDB-C_{18} column (4.6 × 250 mm) fitted with Agilent XDB-UV detector. Sugar identification was done by comparison with reference sugars (L-arabinose, L-fucose, D-xylose, D-mannose, D-galactose, D-glucose, D-glucuronic acid, D-galacturonic acid and D-glucosamine). Calculation of the molar ratio of the monosaccharide was carried out on the basis of the peak area of the monosaccharide (Chen et al., 2012).

FP2-1S and FP2-1P were also hydrolyzed with 2 mol/L trifluoroacetic acid at 100 °C for 6 h, respectively. The complete hydrolysates were analyzed by Whatman 3MM paper chromatography and developing with the solvent system of 1-butanol:pyridine:water (6:4:3) (Harada & Maeda, 1998). The monosaccharide was obtained by extracting with water and its optical rotation value was measured at 20 °C with a P-1020 digital polarimeter, using a 10-cm light path length at 589 nm (Mao et al., 2008).

2.7. Methylation analysis

Methylation analysis was performed as described by Ciucanu and Kerek (1984). In the assay, 2 mg of sample was dissolved in 1 mL of dimethyl sulfoxide, and then 200 mg of anhydrous NaOH was added. The mixture was stirred at room temperature for 1.5 h. A volume of 0.5 mL of iodomethane was added and the mixture was stirred for a further 1.5 h. The reaction was terminated by the addition of water, and the resulting solution was extracted with $CHCl_3$. The extract was washed with distilled water and evaporated to dryness. The completion of methylation was confirmed by Fourier-transform infrared (FTIR) spectroscopy by the disappearance of OH bands. After hydrolysis with 2 mol/L trifluoroacetic acid at 105 °C for 6 h, the methylated sugar residues were converted to partially methylated alditol acetates by reduction with $NaBH_4$, followed by acetylation with acetic anhydride. The derivatized sugar residues were extracted into dichloromethane and evaporated to dryness and dissolved in 100 μL of dichloromethane. The products were analyzed by gas chromatography–mass spectrometry (GC–MS) on a DB 225 using a temperature gradient of 100–220 °C with heating at a rate of 5 °C/min, and a maintenance of a temperature of at 220 °C for 15 min. GC–MS was performed on an HP6890II instrument. Identification of partially methylated alditol acetates was carried out on the basis of retention time and mass fragmentation patterns.

2.8. Spectroscopy analysis

For FTIR spectroscopy, the polysaccharide was mixed with KBr powder, ground up and pressed into 1-mm pellets for measurement in the frequency range of 4000–500 cm^{-1}. FTIR spectra of the polysaccharide were recorded on a Nicolet Nexus 470 spectrometer.

^1H NMR and ^{13}C NMR spectra were recorded at 23 °C using a JEOL JNM-ECP 600 MHz spectrometer. 40 mg of polysaccharide was dissolved in 1 mL of 99% D_2O followed by freeze-dried. The process was repeated twice, and the final sample was dissolved in 1.0 mL of 99.98% D_2O. Chemical shifts are expressed in ppm using acetone as internal standard at 2.225 ppm for ^1H and 31.07 ppm for ^{13}C. ^1H–^1H correlated spectroscopy (COSY), ^1H–^{13}C heteronuclear multiple quantum coherence spectroscopy (HMQC) and ^1H–^{13}C heteronuclear multiple bond correlation spectroscopy (HMBC) experiments were also carried out.

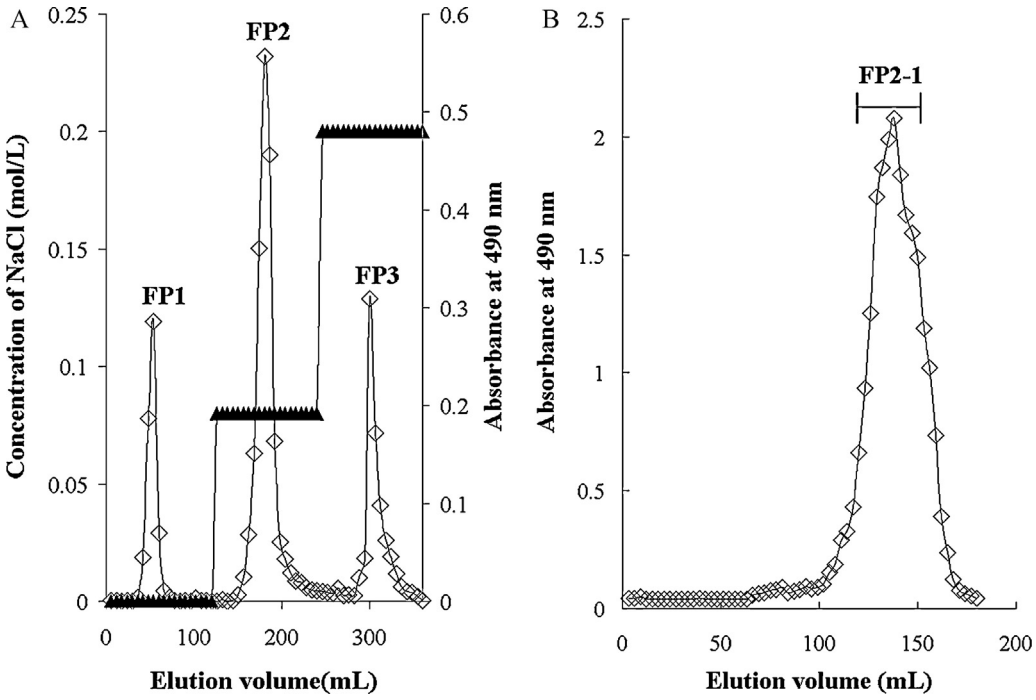

Fig. 1. Preparation of the extracellular polysaccharide produced by coral-associated fungus *P. commune*. (A) The crude polysaccharides obtained from *P. commune* were fractionated using a Q Sepharose Fast Flow column and eluted with a step-wise gradient of 0, 0.08 and 0.2 mol/L NaCl. The fraction eluted with 0.08 mol/L NaCl was pooled, and named as FP2, and (B) FP2 was loaded to a Sephacryl S-100 column and eluted with 0.2 mol/L NH_4HCO_3. The peak fractions containing the polysaccharides were pooled and named as FP2-1.

3. Results and discussion

3.1. Preparation of the extracellular polysaccharide FP2-1 from the coral-associated fungus P. commune

The coral-associated fungus *P. commune* produces extracellular polysaccharides when grown in potato dextrose agar medium. The yield of crude extracellular polysaccharide from the fermented broth was about 0.43 g/L. The crude extracellular polysaccharide was isolated using a Q Sepharose Fast Flow column, eluted with a step-wise gradient of 0, 0.08 and 0.2 mol/L NaCl, into three fractions (Fig. 1A). Among the fractions, FP2 eluted with 0.08 mol/L NaCl was the most abundant and was further purified by a Sephacryl S-100 column (Fig. 1B). A polysaccharide fraction FP2-1 was obtained, and the yield of FP2-1 from crude polysaccharide was about 36%.

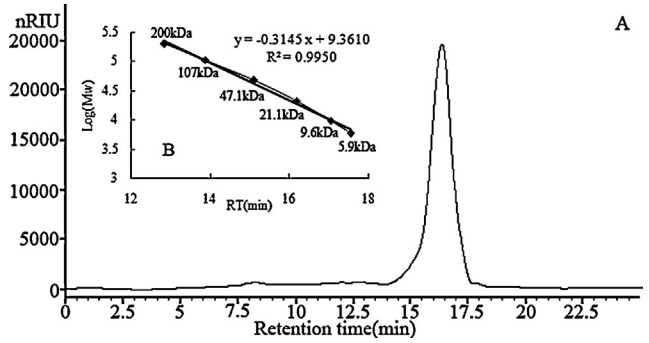

Fig. 2. HPGPC chromatogram of the extracellular polysaccharide FP2-1 and the standard curve of molecular weight. (A) HPGPC chromatogram of FP2-1; (B) the standard curve of molecular weight.

3.2. Chemical composition of FP2-1

FP2-1 appeared as a single and symmetrical peak in the HPGPC chromatogram (Fig. 2A), indicating its homogeneity. On the basis of calibration curve made by dextran standards (Fig. 2B), the molecular weight of FP2-1 was estimated as 18.3 kDa. Chemical analyses showed that FP2-1 contained 96.4% total carbohydrate, with minor amounts of protein (1.23%). No sulfate ester and uronic acid were detected. Reversed-phase HPLC analysis demonstrated that FP2-1 was composed of galactose, mannose and glucose in a molar ratio of 3.9:1.9:1.0. The optical rotations of the galactose and mannose hydrates were +80.3° and +14.1°, respectively, the values were in agreement with those for D-galactose and D-mannose.

The FTIR spectrum of FP2-1 was listed in Fig. 3. The intense and broad signal at 3382 cm^{-1} was the result of valent vibrations OH groups and valent vibration of H_2O constitutional molecules. The signal at 2929 cm^{-1} was attributed to the stretch vibration of the C—H bond. The band at 1649 cm^{-1} was assigned to the bending

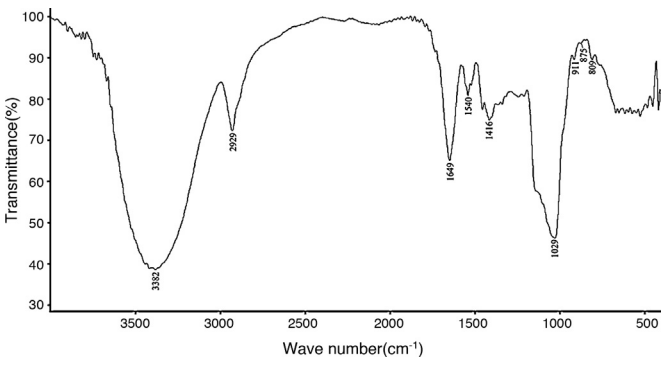

Fig. 3. IR spectrum of FP2-1.

Table 1
Identification of the O-methylated alditol acetates derived from FP2-1.

Methylated alditol acetate	Mass fragments (*m*/*z*)	Molar ratio	Linkage pattern
1,5-Di-O-acetyl-2,3,4,6-tetra-O-methyl-gluctitol	101,117,129,145,161,205	1.96	Glc*p*-(1→
1,2,4-Tri-O-acetyl-3,5,6-tri-O-methyl-galactitol	89,129,139,189,305	2.09	→2)-Gal*f*-(1→
1,4,6-Tri-O-acetyl-2,3,5-tri-O-methyl-galactitol	87,101,117,127,143,159,201,233	6.07	→6)-Gal*f*-(1→
1,2,5-Tri-O-acetyl-3,4,6-tri-O-methyl-mannitol	87,101,129,161,189	1.04	→2)-Man*p*-(1→
1,2,5,6-Tetra-O-acetyl-3,4-di-O-methyl-mannitol	87,129,189,233	1.00	→2,6)-Man*p*-(1→

vibrations of HOH, and the signal at 1540 cm^{-1} was attributed to the vibration of C—O (Mitić, Cakić, & Nikolić, 2010). The band at 1416 cm^{-1} was assigned to the bending vibrations of O—H bond. The signal at 1029 cm^{-1} was due to the stretch vibration of C—O—C linkages. The signal at 911 cm^{-1} was attributed to the asymmetric telescopic vibration of glucopyranose ring (Wu, Hu, Pan, Zhou, & Zhou, 2007). The characteristic absorption signal at 875 cm^{-1} suggested the presence of β-galactofuranose (Ahrazem et al., 2000). In addition, the characteristic absorption at 809 cm^{-1} indicated the presence of α-anomeric configuration of the mannose units (Mathlouthi & Koenig, 1986).

3.3. Methylation analysis

The identification and the proportions of the methylated alditol acetates of FP2-1 were listed in Table 1. Methylation analysis could provide important information for the linkage position assignments of each monosaccharide. Large amounts of 1,4,6-tri-O-acetyl-2,3,5-tri-O-methyl-galactitol, and small amounts of 1,2,4-tri-O-acetyl-3,5,6-tri-O-methyl-galactitol, 1,5-di-O-acetyl-2,3,4,6-tetra-O-methyl-glucitol and 1,2,5-tri-O-acetyl-3,4,6-tri-O-methyl-mannitol were detected in FP2-1, suggesting that FP2-1 was mainly composed of (1→6)-linked galactofuranose with small amounts of (1→2)-linked galactofuranose, (1→)-linked glucopyranose and (1→2)-linked mannopyranose residues. Moreover, FP2-1 should have a partially branched structure because of the presence of the 1,2,5,6-tetra-O-acetyl-3,4-di-O-methyl-mannitol, which originated from (1→2,6)-linked mannopyranose residues. The molar ratios of (1→6)-linked galactofuranose, (1→2)-linked galactofuranose, (1→)-linked glucopyranose, (1→2)-linked mannopyranose and (1→2,6)-linked mannopyranose residues were about 6.07:2.09:1.96:1.04:1.00. The results suggested that FP2-1 was a branched glucomannogalactan. The monosaccharide composition determined by HPLC showed a lower galactose and higher mannose content in comparison with the results of methylation analysis. The latter value may be the result of underestimation of the mannose derivatives, and it may be due to loss of the degradation products during water washing of the derivatized samples.

3.4. Analysis of NMR spectroscopy

In the ^{1}H NMR spectrum of FP2-1 (Fig. 4A), five major proton signals occurred at 5.21, 5.20, 5.10, 5.08 and 5.04 ppm. Other proton signals located at 3.4–4.3 ppm were assigned to H2–H6 of the sugar residues. In the anomeric region of the ^{13}C NMR spectrum (Fig. 4B) of FP2-1 showed five anomeric carbon signals at 109.40, 107.80, 103.52, 99.60 and 99.50 ppm. The signals at 109.40 and 107.80 ppm were attributed to C-1 of β-galactofuranose units because of extremely low field shifts (Ahrazem et al., 2007; Nagaoka et al., 1996), while the signals at 103.52, 99.60 and 99.50 ppm might be the signals of the mannopyranose and glucopyranose linkages (Tischer, Gorin, De Souza, & Barreto-Bergter, 2002). The signal appearing at 88.09 ppm was due to C-2 substituted galactofuranose residues (Ahrazem, Prieto, Leal, Jiménez-Barbero, & Bernabé, 2002). In the DEPT spectrum, the signal at 70.68 ppm was assigned to the substituted C-6 of mannose and galactose units (Ahrazem et al., 2007; Giménez-Abián, Bernabé, Leal, Jiménez-Barberoa, & Prieto, 2007). The result confirmed the presence of the substituted C-6 linkage patterns, which was in agreement with methylation results.

^{1}H–^{1}H COSY spectrum (Fig. 4C) of FP2-1 gave anomeric H1–H2 assignments of the sugar residues. The ^{1}H–^{13}C HMQC spectrum (Fig. 4D) of FP2-1 also permitted assignment of the anomeric relevant C—H signals. The anomeric proton signal of A was correlated to the anomeric carbon signal at 107.80 ppm, thus A was assigned to the (1→2)-linked β-galactofuranose residue (Bernabe, Salvachua, Jiménez-Barbero, Leal, & Prieto, 2011; Cordeiro et al., 2012). The anomeric proton signal of B was correlated to the anomeric carbon signal at 99.50 ppm. Combined with methylation analysis and the chemical shift data of similarly substituted sugar residues (Purama, Goswami, Khan, & Goyal, 2009), B was attributed to the (1→)-linked α-glucopyranose residue. The anomeric proton signal of C was related to the anomeric carbon signal at 109.40 ppm, and C was attributed to the (1→6)-linked β-galactofuranose residue (Ahrazem et al., 2007). The anomeric proton signal of D was related to the anomeric carbon signal at 99.60 ppm, and the anomeric proton signal of E was correlated to the anomeric carbon signal at 103.52 ppm. The ^{1}H–^{13}C HMQC spectrum revealed the substitution of D at C-2 and C-6 due to the downfield shifts of the C-2 (78.45 ppm) and C-6 (70.68 ppm) as compared with that of the parent α-mannopyranose residue (Chen et al., 2012), thus D was assigned to be (1→2,6)-linked α-mannopyranose residue. E was attributed to the (1→2)-linked α-mannopyranose residue because of the downfield shift (78.45 ppm) of the resonance of C-2 in comparison with that of the parent α-mannopyranose residue.

In the ^{1}H–^{13}C HMBC spectrum (Fig. 4E) of FP2-1, the presence of cross signal H-1/C-4 in A and C illustrated that both of A and C were β-galactofuranose configuration. The cross signal H-1/C-6 of C suggested that C was (1→6)-β-Gal*f*. The cross signal of H-1 (B)/C-2 (A) indicated the presence of the linkage α-Glc*p*-(1→2)-β-Gal*f*-(1→. From the cross signal of H-1(A)/C-6(C), it was presumed that (1→2)-β-Gal*f* linked to the C-6 of (1→6)-β-Gal*f*. The presence of cross signal H-1 (C)/C-6 (D) suggested that the H-1 of (1→6)-β-Gal*f* was connected to the C-6 site of (1→2,6)-α-Man*p*.

These analyses allowed the identification of most of the ^{1}H and ^{13}C signals of the sugar residues (Table 2). The NMR results were in agreement with the methylation results. Thus FP2-1 was deduced to consist of a backbone of (1→2)-linked α-mannopyranose with the every second residue substituted at position 6 by a pentasaccharide branch. The branches consist of four (1→6)-linked β-galactofuranose residues with terminal α-glucopyranose residue attached to the last galactofuranose residue at position 2.

FP2-1 is a novel galactofuranose-containing glucomannogalactan differing from previously described extracellular polysaccharides (Shibata & Okawa, 2011). Some galactofuranose-containing polysaccharides from fungi have been reported (Prieto, Ahrazem, Bernabé, & Leal, 2004). Generally, the polysaccharides produced by *Penicillium* and *Aspergillus* sp. contained a long chain of linear (1→5)-linked β-galactofuranan. But the polysaccharides from *P. corylophilum* and *P. erubescens* had additional branches at C-6 of the chain by (1→)-linked β-galactofuranose units (Leal et al., 1997). Recently, a galactofuranose-containing extracellular polysaccharide Ps1-1 was obtained from the

Table 2
^{1}H and ^{13}C chemical shifts for the extracellular polysaccharide FP2-1.

Residues[a]	Chemical shifts (ppm)[b]					
	H1/C1	H2/C2	H3/C3	H4/C4	H5/C5	H6/C6
A	5.20/107.80	4.21/88.09	4.28/77.70	4.08/82.57	3.76/73.89	3.85,3.69/64.28
B	5.10/99.50	3.61/72.40	3.76/74.30	3.47/68.39	3.74/72.65	3.91,3.80/62.10
C	5.08/109.40	4.16/82.57	4.10/78.45	4.05/84.70	4.00/71.23	3.93,3.68/70.68
D	5.04/99.60	3.88/78.45	4.02/71.21	3.68/68.36	3.93/71.28	3.79,3.47/70.68
E	5.21/103.52	3.95/78.45	3.89/72.38	3.69/68.21	3.76/72.36	3.86,3.75/62.52

[a] A–E correspond to (1→2)-β-Gal*f*, (1→)-α-Glc*p*, (1→6)-β-Gal*f*, (1→2,6)-α-Man*p* and (1→2)-α-Man*p*, respectively. Gal*f*: galactofuranose, Man*p*: mannopyranose, Glc*p*: glucopyranose.
[b] The spectra were recorded using a JEOL JNM-ECP 600 MHz spectrometer. Chemical shifts are referenced to internal acetone at 2.225 ppm for ^{1}H and 31.07 ppm for ^{13}C.

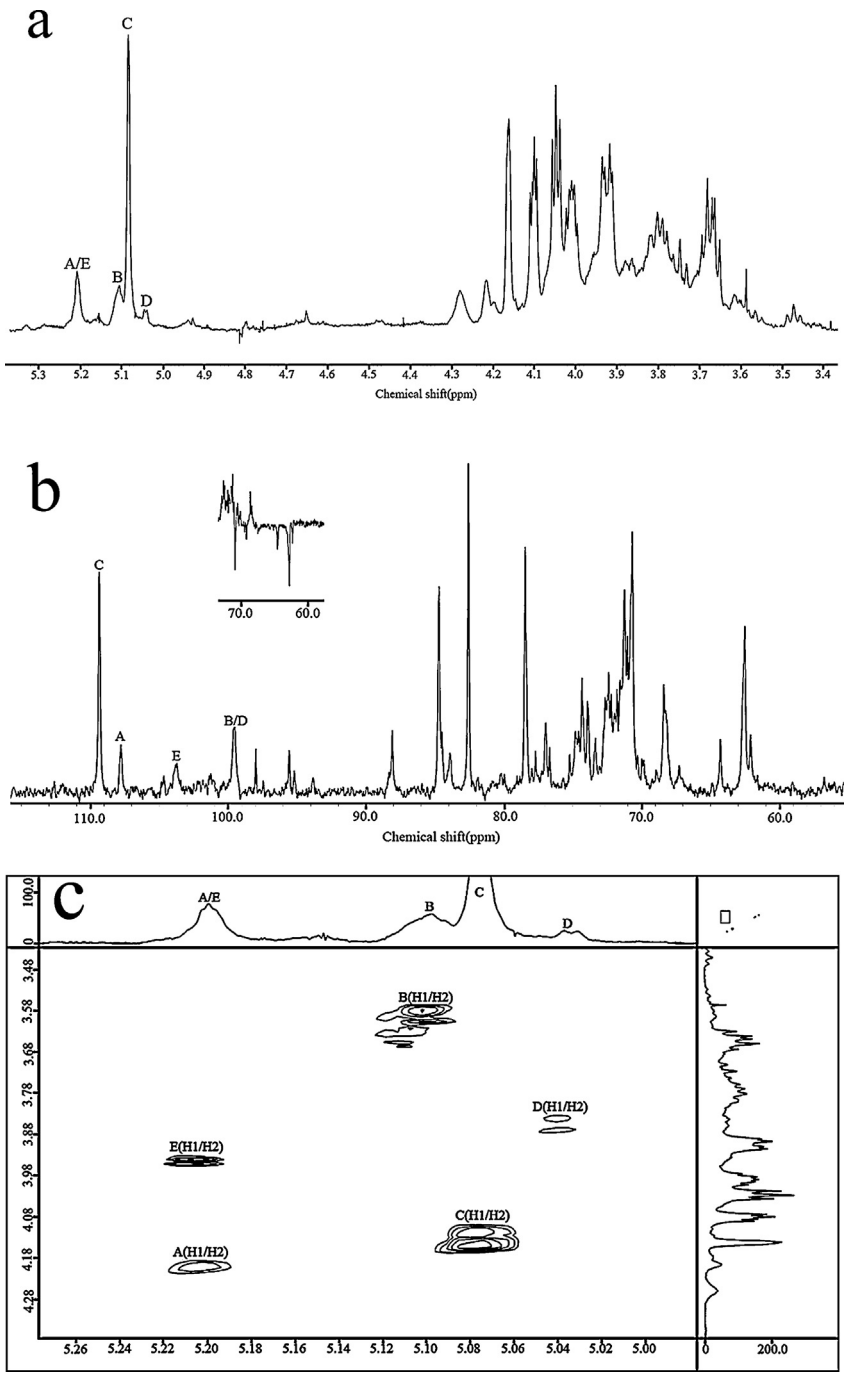

Fig. 4. NMR spectra of the extracellular polysaccharide FP2-1. Spectra were performed at 23 °C on a JEOL ECP 600 MHz spectrometer using acetone as internal standard. (A) ^{1}H NMR spectrum; (B) ^{13}C NMR and DEPT spectra; (C) ^{1}H–^{1}H COSY spectrum; (D) ^{1}H–^{13}C HMQC spectrum; (E) ^{1}H–^{13}C HMBC spectrum. A–E correspond to (1→2)-β-Gal*f*, (1→)-α-Glc*p*, (1→6)-β-Gal*f*, (1→2,6)-α-Man*p* and (1→2)-α-Man*p*, respectively. Gal*f*: galactofuranose, Man*p*: mannopyranose, Glc*p*: glucopyranose.

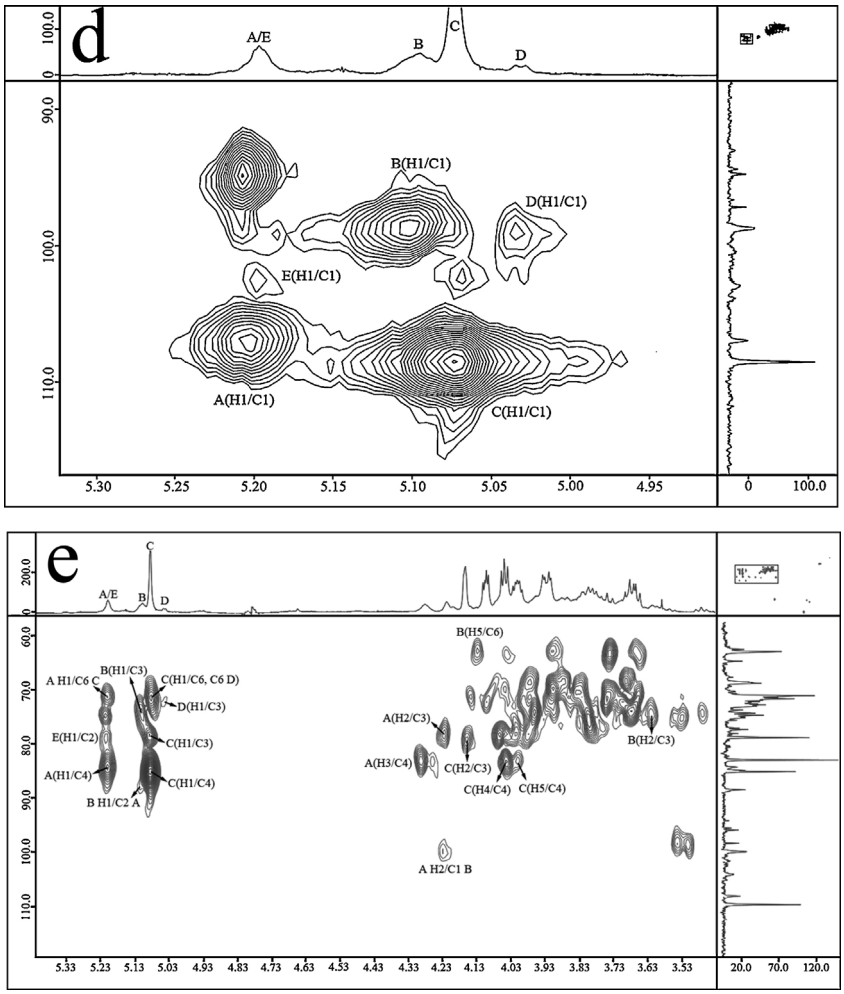

Fig. 4. (*Continued*).

deep-sea fungus *P. griseofulvum*. Ps1-1 consisted of a long chain of [→5)-β-galactofuranose-(1→5)-β-galactofuranose-(1→]$_n$, with about 20% of (1→)-linked β-galactofuranose residues and phosphate esters linked to the C-6 of (1→5)-linked β-galactofuranose residues (Chen et al., 2013). The structure of FP2-1 was different from those of the polysaccharides. Compared with the Ps1-1, the galactofuranose chain of FP2-1 was composed of [→2)-β-galactofuranose-(1→6)-β-galactofuranose-(1→6)-β-galactofuranose-(1→6)-β-galactofuranose-(1→]. The present result suggested that symbiotic and epiphytic microorganisms could be a potential source of extracellular polysaccharides with unique structures. Some investigations demonstrated that galactofuranose metabolism could be a very attractive candidate as a target for new antimicrobial drugs (Pedersen & Turco, 2003), and galactofuranose-containing polysaccharides to be worthy of further study (Shibata, Saitoh, Tadokoro, & Okawa, 2009).

4. Conclusion

The extracellular polysaccharide FP2-1 produced by the coral-associated fungus *P. commune* is a galactofuranose-containing glucomannogalactan. On the basis of detailed NMR spectroscopic analyses, FP2-1 was characterized to contain a backbone of (1→2)-linked α-mannopyranose residues with side chains built up of galactofuranose residues with terminal glucopyranoses. FP2-1 was a novel extracellular polysaccharide with a structure different from those of other extracellular polysaccharides.

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