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# Structural elucidation of the exopolysaccharide produced by the mangrove fungus *Penicillium solitum*

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ABSTRACT

A water soluble extracellular polysaccharide, designated GW-12, was obtained from the liquid culture broth of the mangrove fungus *Penicillium solitum* by ethanol precipitation, anion-exchange and size-exclusion chromatography. Reversed-phase high performance liquid chromatography analysis showed that GW-12 mainly consisted of D-mannose, and its molecular weight was estimated to be about 11.3 kDa determined by high performance gel permeation chromatography. On the basis of chemical and spectroscopic analyses, including methylation analysis and nuclear magnetic resonance (NMR) spectroscopy, the structure of GW-12 may be represented as a mannan with branches. The main chain of GW-12 was composed of (1→2)-linked α-D-mannopyranose and (1→6)-linked α-D-mannopyranose residues, branched by single α-D-mannopyranose units attached to the main chain at C-6 positions of (1→2)-linked α-D-mannopyranose residues. There was three branch points for every seven sugar residues in the backbone.



## 1. Introduction

Marine microorganisms are recognized as an abundant source of bioactive substances (Kobayashi & Tsuda, 2004; Peng et al., 2012). In recent years, the interest in extracellular polysaccharides produced by marine fungi is increasing (Sun et al., 2009; Wang et al., 2013). The diversity of marine fungi determines the diversity of extracellular polysaccharides (Raghukumar, 2008). The extracellular polysaccharides produced by marine fungi hold a great potential application in biology and pharmacology (Selbmann, Onofri, Fenice, Federici, & Petruccioli, 2002).

Mangrove forest is considered a dynamic ecotone or transition zone between terrestrial and marine habitats, and is biodiversity spots for marine fungi (Gopal & Chauhan, 2006; Shearer et al., 2007). Mangrove fungi constitute the second largest ecological group of the marine fungi due to its complex and special environment (Sridhar, 2004). So far, few reports on the extracellular polysaccharides from mangrove fungi could be found (Chen et al., 2011). With today's interest in new renewable sources of chemicals and polymers, the extracellular polysaccharides isolated from mangrove fungi are recognized as a potential source to be explored. In this study, a homogenous extracellular polysaccharide from the liquid culture broth of the mangrove fungus *Penicillium solitum* was isolated, and its structural characterizations were elucidated by a combination of chemical and spectroscopic methods, including gas chromatography–mass spectrometry (GC–MS), one- and two-dimensional nuclear magnetic resonance (1D and 2D NMR) spectroscopy.

## 2. Materials and methods

### 2.1. Materials

Monosaccharides (D-glucose, D-mannose, D-galactose, L-rhamnose, D-xylose, L-arabinose, L-fucose, D-glucuronic acid, D-galacturonic acid and D-glucosamine), 1,1-diphenyl-2-picrylhydrazyl, trifluoroacetic acid, thiobarbituric acid, 1-phenyl-3-methyl-5-pyrazolone, L-cysteine methyl ester and *o*-tolyl isothiocyanate were from Sigma–Aldrich (St. Louis, MO, USA). Pullulan standards ($M_w$: 47.1, 21.1, 9.6, 5.9 and 1.0 kDa) were from Showa Denko K.K. (Tokyo, Japan). Dialysis membranes (flat width 44 mm, molecular weight cut-off 3500) were from Lvniao (Yantai, China). Q Sepharose Fast Flow and Sephacryl S-100 were from GE Healthcare Life Sciences (Piscataway, NJ, USA).

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### 2.2. Microbial strain and culture conditions

The fungus *P. solitum* was separated from the soil of *Rhizophora stylosa* collected from the mangrove of the gulf of Shankouyingluo, Guangxi, China (N: 21° 29′ 798″ E: 109° 45′ 470″). It was identified according to its morphological characteristics and internal transcribed spacer (ITS) rDNA sequences, and the accession number of Genbank was KJ577599. The extracellular polysaccharide producing strain was activated on potato dextrose agar slants and stored at 25 °C for 3 days. *P. solitum* was then grown in the liquid medium containing potato steep liquor (20 g/L), maltose (2 g/L), mannitol (2 g/L), glucose (1 g/L), monosodium glutamate (0.5 g/L), peptone (0.5 g/L) at 25 °C for 25 days on a reciprocal shaker. The cultivation experiment was performed in 1000 mL of Erlenmeyer flasks containing 300 mL of the medium in a vessel. Finally, 70 L of liquid culture broth were obtained.

### 2.3. Isolation and purification of the extracellular polysaccharide

The liquid culture broth was filtered through cheesecloth to separate the mycelium and supernatant. The supernatant was concentrated under reduced pressure at 40 °C, and precipitated by adding fourfold of the volume of 95% ethanol (v/v) and was kept at 4 °C for 24 h. The precipitate was collected by centrifugation at 3600 × *g* for 10 min, and dialyzed in a cellulose membrane (flat width 44 mm, molecular weight cut off 3500) against distilled water for 48 h. The retained fraction was vacuum-dried, and the protein in the fraction was removed as described by Sevag, David, and Smolens (1938). The crude polysaccharide was fractionated with a Q Sepharose Fast Flow column (300 mm × 30 mm) by eluting with a step gradient of 0–2 mol/L NaCl. The fractions were assayed for carbohydrate content by the phenol–sulfuric acid method (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956). The fractions eluted with distilled water and 0.05 mol/L NaCl were pooled, dialyzed and further purified on a Sephacryl S-100 column (100 cm × 1.6 cm) with 0.2 mol/L $NH_4Cl$ as eluent. The major polysaccharide fraction was pooled and evaporated to dryness.

### 2.4. Analytical techniques

Total sugar content was measured by the phenol–sulfuric acid method using mannose as the standard (Dubois et al., 1956). Protein content was assayed according to the method of Bradford (1976) using bovine serum albumin as the standard. Sulfate content was measured according to Therho and Hartiala (1971). Amino sugar content was assayed according to the method of Wagner (1979). Uronic acid content was determined by the carbazole–sulfuric acid method using glucuronic acid as standard (Blumenkrantz & Asboe-Hansen, 1973). Purity and molecular weight was assessed by high performance gel permeation chromatography (HPGPC) on a Shodex OHpak SB-803 HQ column (8.0 mm × 300 mm), and the column calibration was performed with pullulan standards (Li et al., 2011).

### 2.5. Analysis of monosaccharide composition

Monosaccharide composition was determined by reversed-phase high performance liquid chromatography (HPLC) after pre-column derivatization (Qi et al., 2012). Briefly, 2 mg of polysaccharide was hydrolyzed with 2 mol/L trifluoroacetic acid at 105 °C for 6 h. Excess acid was removed by co-distillation with methanol after the hydrolysis. The dry hydrolysate was dissolved in 100 μL of 0.3 mol/L NaOH, and added to 120 μL of 0.5 mol/L methanol solution of 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, and 10 μL of the resulting solution was injected into the Eclipse XDB-$C_{18}$ column (4.6 mm × 250 mm). The chromatograms were performed on an Agilent 1260 Infinity HPLC instrument fitted with Agilent XDB-UV detector (254 nm). The mobile phase was a mixture of 0.1 mol/L $KH_2PO_4$ (pH 6.7) – acetonitrile (83:17). Sugar identification was done by comparison with reference sugars (L-rhamnose, L-arabinose, L-fucose, D-xylose, D-mannose, D-galactose, D-glucose, D-glucuronic acid, D-galacturonic acid and *N*-acetyl-β-D-glucosamine). Calculation of the molar ratio of the monosaccharide was carried out on the basis of the peak area of the monosaccharide.

### 2.6. Determination of sugar configuration

Sugar configuration was determined as described by Tanaka, Nakashima, Ueda, Tomii, and Kouno (2007). 5 mg of polysaccharide was hydrolyzed with 2 mol/L trifluoroacetic acid at 105 °C for 6 h. Excess acid was removed with methanol in a rotary evaporator. The hydrolysate was heated with L-cysteine methyl ester in pyridine at 60 °C for 60 min. A solution of the *o*-tolyl isothiocyanate was added to the mixture, and was further heated at 60 °C for 60 min. The reaction mixture was directly analyzed on an Agilent 1260 Infinity HPLC instrument using an Eclipse XDB-$C_{18}$ column (4.6 mm × 250 mm) and detected by an Agilent XDB-UV detector at 250 nm. Sugar configuration was identified by comparison with reference sugars.

### 2.7. Methylation analysis

Methylation analysis was performed by the method of Hakomori (1964). In brief, polysaccharide in dimethyl sulfoxide was methylated using NaH and iodomethane, and the completeness of methylation was confirmed by infrared spectroscopy. 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 derivatised sugar residues were extracted into dichloromethane and evaporated to dryness, and dissolved again in 100 μL of dichloromethane. The products were analyzed by gas chromatography–mass spectrometry (GC–MS) on a HP6890II/5973 instrument using a DB 225 fused silica capillary column (0.25 mm × 30 m) (Agilent Technologies Co. Ltd., USA). The temperature was increased from 100 to 220 °C at a rate of 5 °C/min then maintained at 220 °C for 15 min. Identification of partially methylated alditol acetates was carried out on the basis of the retention time and its mass fragmentation patterns.

### 2.8. IR and UV spectroscopy analysis

The Fourier-transform infrared (FTIR) spectrum of the polysaccharide was measured on a Nicolet Nexus 470 spectrometer. The polysaccharide was mixed with KBr powder, ground and pressed into a 1 mm pellets for FTIR measurements in the frequency range of 4000–400 $cm^{-1}$. Ultraviolet (UV) spectrum was recorded on a UV-2102 PCS spectrophotometer between 190 and 400 nm.

### 2.9. NMR spectroscopy analysis

50 mg of polysaccharide was deuterium exchanged by two successive freeze-drying steps in 99% $D_2O$ and then dissolved in 0.5 mL of 99.98% $D_2O$. $^1H$ nuclear magnetic resonance (NMR) and $^{13}C$ NMR spectra were measured at 23 °C using a JEOL JNM-ECP 600 MHz spectrometer. $^1H$–$^1H$ correlated spectroscopy (COSY), $^1H$–$^{13}C$ heteronuclear multiple quantum coherence spectroscopy (HMQC)

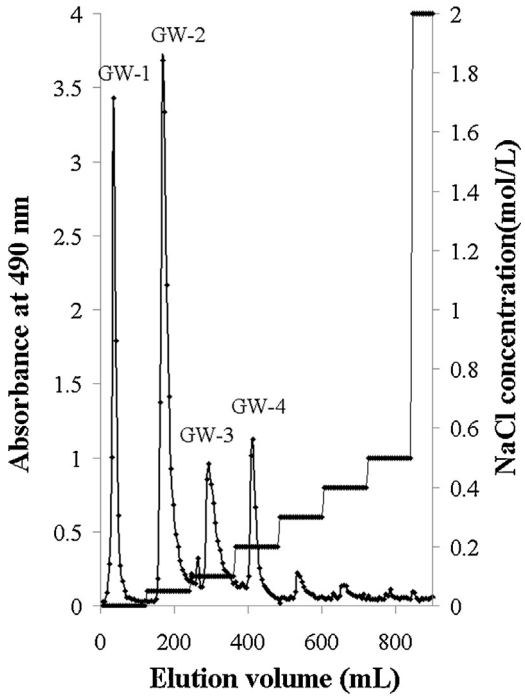


**Fig. 1.** Isolation of the crude extracellular polysaccharide from the liquid culture broth of the mangrove fungus *P. solitum* on a Q Sepharose Fast Flow column. The four polysaccharide fractions were obtained and termed as GW-1, GW-2, GW-3 and GW-4, respectively.

and $^{1}H$–$^{13}C$ heteronuclear multiple bond correlation spectroscopy (HMBC) experiments were also carried out. Chemical shifts are expressed in ppm using acetone as internal standard at 2.225 ppm for $^{1}H$ and 31.07 ppm for $^{13}C$.

## 3. Results and discussion

### 3.1. Purification and composition analysis of the extracellular polysaccharides

The crude extracellular polysaccharide was obtained from the liquid culture broth of the mangrove fungus *P. solitum*, and its yield was about 0.55 g/L. The crude extracellular polysaccharide was fractionated using a Q Sepharose Fast Flow column (Fig. 1). This fractionation process resulted in four polysaccharide fractions that were termed GW-1, GW-2, GW-3 and GW-4. Of the fractions, GW-1 and GW-2 were more abundant and further purified on a Sephacryl S-100 column. Finally, two polysaccharide fractions, designated GW-12 and GW-22, were obtained. The yield of GW-12 and GW-22 from crude extracellular polysaccharide was about 31.0% and 28.2%, respectively.

As shown in Fig. 2, GW-12 and GW-22 appeared as single and symmetrical peaks in the HPGPC chromatogram, respectively, indicating that they could be homogeneous polysaccharides. The retention times in HPGPC chromatogram of GW-12 and GW-22 were used to calculate their molecular weights by the obtained regression equation. Thus, the molecular weights of GW-12 and GW-22 were estimated as about 11.3 kDa and 13.9 kDa, respectively. Monosaccharide compositions analysis by reversed-phase HPLC after pre-column derivatization showed that GW-12 and GW-22 mainly consisted of mannose. No uronic acid, amino sugars and sulfate ester were detected in the two polysaccharides. GW-12 did not contain protein, while the protein content of GW-22 was up to 13.28%. From the UV spectrum of GW-12, no absorption was found at 280 nm, which further indicated that no protein was present in

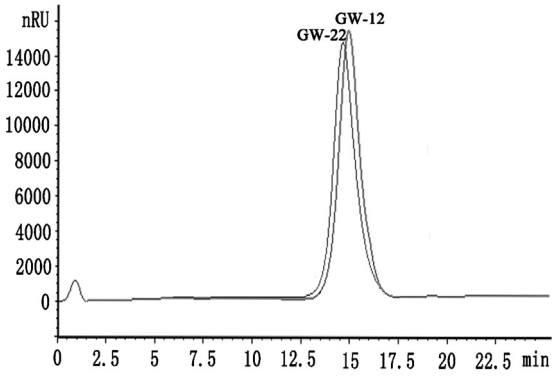


**Fig. 2.** HPGPC chromatogram of GW-12 and GW-22 on a Shodex OHpak SB-803 HQ column (8.0 mm × 300 mm).

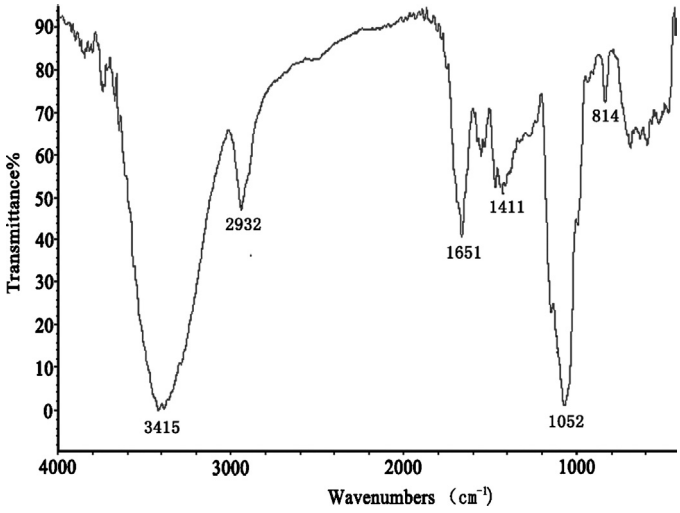


**Fig. 3.** IR spectrum of the extracellular polysaccharide GW-12.

GW-12. Here, GW-12 was chosen to further structural analysis. Reversed-phase HPLC analysis of the reaction mixture of GW-12 exhibited a peak at 9.41 min, which coincided with the derivative of D-mannose, identifying the absolute configuration of the sugar component.

### 3.2. Methylation analysis

Methylation analysis could provide important information for the linkage pattern of the sugar residues. The identification and the proportions of the methylated alditol acetates of GW-12 were listed in Table 1. A large amount of 1,5-di-O-acetyl-2,3,4,6-tetra-O-methyl-D-mannitol was detected in GW-12, indicating the presence of (1→)-linked mannopyranose residues. 1,2,5-tri-O-acetyl-3,4,6-tri-O-methyl-D-mannitol and 1,5,6-tri-O-acetyl-2,4,6-tri-O-methyl-D-mannitol were also found, suggesting that GW-12 was composed of (1→2)-linked mannopyranose and (1→6)-linked mannopyranose residues. In addition, GW-12 should have a partially branched structure due to the presence of 1,2,5,6-tetra-O-acetyl-3,4-di-O-methyl-D-mannitol, which originated from the (1→2,6)-linked mannopyranose residues. Thus, GW-12 was a mannan with branches. The molar ratios of the (1→)-linked mannopyranose, (1→2)-linked mannopyranose, (1→6)-linked mannopyranose and (1→2,6)-linked mannopyranose residues were 1.6:1.1:1.0:1.5 in GW-12.

**Table 1**
Result of methylation analysis of the extracellular polysaccharide GW-12.

| Methylated sugar[a] | Major mass fragments (*m*/*z*) | Molar ratio | Deduced linkage pattern |
|---|---|---|---|
| 2,3,4,6-$Me_4$-Man | 87, 101, 117, 129, 145, 161, 205 | 1.6 | Man*p*-(1→ |
| 3,4,6-$Me_3$-Man | 129, 161, 189 | 1.1 | →2)-Man*p*-(1→ |
| 2,3,4-$Me_3$-Man | 87, 101, 117, 129, 161, 189, 233 | 1.0 | →6)-Man*p*-(1→ |
| 3,4-$Me_2$-Man | 129, 189 | 1.5 | →2,6)-Man*p*-(1→ |

[a] 2,3,4,6-$Me_4$-Man: 1,5-di-O-acetyl-2,3,4,6-tetra-O-methyl-mannitol; 3,4,6-$Me_3$-Man: 1,2,5-tri-O-acetyl-3,4,6-tri-O-methyl-mannitol; 2,3,4-$Me_3$-Man:1,5,6-tri-O-acetyl-2,3,4-tri-O-methyl-mannitol; 3,4-$Me_2$-Man: 1,2,5,6-tetra-O-acetyl-3,4-di-O-methyl-mannitol.

### 3.3. IR analysis

As shown in the FTIR spectrum (Fig. 3), the broad and intense band at 3415 $cm^{-1}$ was the stretch vibration of hydroxyl groups. The signal at 2932 $cm^{-1}$ was attributed to the stretch vibration of the C—H bond, and the band at 1651 $cm^{-1}$ was due to the bending vibration of HOH (Mitić, Cakić, & Nikolić, 2010). The band at 1413 $cm^{-1}$ was assigned to bending vibration of C—H bond, and the band at 1052 $cm^{-1}$ was attributed to the stretch vibration of C—O—C linkages. The strong characteristic absorption at 814 $cm^{-1}$ indicated the presence of α-anomeric configuration of the mannose units (Mathlouthi & Koenig, 1986).

### 3.4. NMR spectroscopy analysis

In the $^1H$ NMR spectrum of GW-12 (Fig. 4A), the anomeric proton signals at 5.08, 5.16, 5.26 and 5.28 ppm in a molar ratio of 1.61:1.53:1.14:1.00 were labeled A, B, C and D, respectively. The anomeric proton signals at 5.08, 5.16, 5.26 and 5.28 ppm were assigned to α-configuration pyranose units. In Fig. 4A, a shoulder was clearly observed in peak B, suggesting two overlapping signals, which probably corresponded to a same type of residue with different environments. Other proton signals were located in the region of 4.30–3.60 ppm, which were assigned to H2–H6 of the sugar residues. As shown in the $^{13}C$ NMR spectrum (Fig. 4B) of GW-12, three anomeric carbon signals occurred at 99.8, 102.2 and 103.8 ppm. The signal occurring at 80.0 and 79.7 ppm were assigned to the C-2 substituted mannose residues. As shown in the DEPT spectrum, the signal at 67.0 ppm was attributed to the substituted C-6 of mannose units, while the signals at 62.6 and 62.5 ppm were assigned to the C-6 non-substituted mannose residues (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.

The $^1H$–$^1H$ COSY spectrum (Fig. 4C) of GW-12 gave the H1–H2 correlations of the sugar residues. The $^1H$–$^{13}C$ HMQC spectrum (Fig. 4D) of GW-12 also permitted assignment of the direct C—H coupling. The anomeric proton signal at 5.08 ppm correlated with the anomeric carbon signal at 103.8 ppm. The proton signals at 5.16, 5.26 and 5.28 ppm were related to the anomeric carbon signals at 99.8, 102.2 and 102.2 ppm, respectively. In Fig. 4D, the signal at 5.16 ppm (peak B) unfolded into two cross peaks at 99.8 and 103.8 ppm, further confirming the presence of the residue B with different environments.

In the $^1H$–$^1H$ COSY spectrum of GW-12, there was a distinguishable difference of chemical shifts of the H-2 of B and C (data not shown). Combined with the $^1H$–$^{13}C$ HMQC spectrum, the signal at 80.0 ppm was attributed to the H-2 of B, and the signal at 79.7 ppm was attributed to the H-2 of C. The $^1H$–$^{13}C$ HMQC spectrum revealed the substitution of B at C-6 due to the downfield shifts of the C-6 (67.0 ppm) as compared with that of parent α-D-mannopyranose residues. Thus B was assigned to be (1→2,6)-linked α-D-mannopyranose residues. A was proposed to be a (1→)-α-D-mannopyranose residues, and C was attributed to the (1→2)-linked α-D-mannopyranose residues. D was assigned to the (1→6)-linked α-D-mannopyranose residues because of the downfield shift (67.0 ppm) of the resonance of C-6 in comparison with that of the parent α-D-mannopyranose residues (Giménez-Abián et al., 2007). Accurate chemical shifts of C-5 of C and D residues could not be confirmed because of serious peak overlapping. Combining with the data from the $^1H$ NMR, $^{13}C$ NMR, $^1H$–$^1H$ COSY and $^1H$–$^{13}C$ HMQC spectra, the assignments of almost all the proton and carbon signals of A–D units were completed (Table 2).

Based on the analysis of the $^1H$–$^{13}C$ HMBC spectrum (Fig. 4E) of GW-12, the repeating units of the monosaccharide sequences in the polysaccharide chain were established. The H-1 signal at 5.26 ppm of the (1→2)-linked α-D-mannopyranose was correlated to the C-2 signal at 80.0 ppm of the (1→2,6)-linked α-D-mannopyranose, and the H-2 signal at 4.06 ppm of the (1→2,6)-linked α-D-mannopyranose was related to the C-1 signal at 102.2 ppm of the (1→2)-linked α-D-mannopyranose. Thus the structure fragment was confirmed as →2)-α-D-Man*p*-(1→2,6)-α-D-Man*p*-(1→. The cross signal of H-1 at 5.28 ppm with the C-2 at 79.7 ppm and the cross signal of H-2 at 4.15 ppm with the C-1 at 102.2 ppm, indicating the linkage →6)-α-D-Man*p*-(1→2)-α-D-Man*p*-(1→. The cross signal of H-1 at 5.16 ppm and C-6 at 67.0 ppm of the (1→6)-linked α-mannopyranose allowed the determination of the fragment as →2,6)-α-D-Man*p*-(1→6)-α-D-Man*p*-(1→. The signal of H-1 at 5.08 ppm of the (1→)-linked α-D-mannopyranose was correlated to the C-6 at 67.0 ppm of the (1→2,6)-linked α-D-mannopyranose, thus the (1→)-linked α-D-mannopyranose was presumed to link to the C-6 of →2,6)-α-D-Man*p*-(1→. Thus, GW-12 was deduced to be a branched mannan. The backbone of GW-12 mainly was composed of (1→2)-linked α-D-mannopyranose and (1→6)-linked α-D-mannopyranose. The branches consisted of terminal α-D-mannopyranose residues. Combined with the relative integrals of the anomeric proton signals obtained from the $^1H$ NMR spectrum and the proportions of the sugar residues from methylation analysis, it could be deduced that there was three branch points for every seven sugar residues in the backbone of GW-12. One of the possible repeating structures of GW-12 was shown in Fig. 5.

The extracellular polysaccharides produced by *Penicillium* species have been reported (Leal et al., 1997; Pessoni, Freshour, Figueiredo-Ribeiro, Hahn, & Braga, 2005; Sun et al., 2009). Ahrazem et al. (1999) found that the cell wall polysaccharide from *Penicillium vermoesenii* have a mannose core consisting of a short chain of α-(1→6)-linked D-mannopyranose. Chen, Mao, Wang, Zhou, et al. (2013) and Chen, Mao, Wang, Zhu, et al. (2013) isolated an extracellular polysaccharide from the deep-sea fungus *Penicillium griseofulvum*, which was composed of a long chain of galactofuranan and a mannose core. Structure of FP2-1 produced by the coral-associated fungus *Penicillium commune* may be represented as a backbone of (1→2)-linked α-D-mannopyranose with the every second residue substituted at position 6 by a pentasaccharide branch (Chen, Mao, Wang, Zhou, et al., 2013; Chen, Mao, Wang, Zhu, et al., 2013). Compared with the extracellular polysaccharides from *Penicillium* sp., GW-12 had different the sequences of sugar residues and the side chain. The branched chain of GW-12 was different from those previously described for mannans from marine microorganisms *Aspergillus* sp. Y16 and *Edwardsiella tarda* (Chen et al., 2011; Guo et al., 2010).

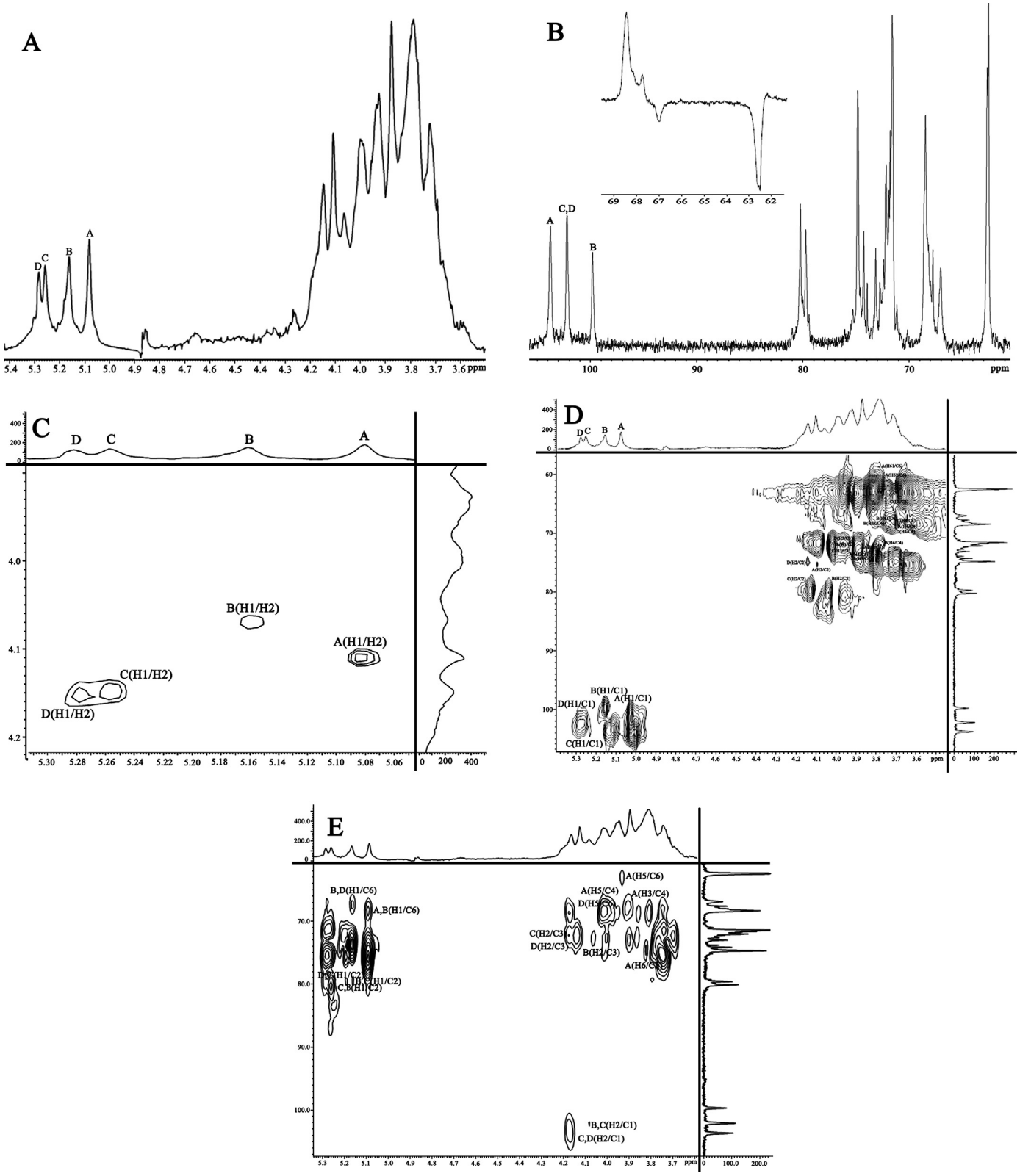


**Fig. 4.** NMR spectra of the extracellular polysaccharide GW-12. Spectra were performed at 23 °C on a JEOL ECP 600 MHz spectrometer using acetone as internal standard. (A) $^{1}$H NMR spectrum; (B) $^{13}$C NMR and DEPT spectra; (C) $^{1}$H–$^{1}$H COSY spectrum; (D) $^{1}$H–$^{13}$C HMQC spectrum; (E) $^{1}$H–$^{13}$C HMBC spectrum. A–D correspond to (1→)-α-D-Man*p*, (1→2,6)-α-D-Man*p*, (1→2)-α-D-Man*p*, (1→6)-α-D-Man*p*, respectively. Man*p*, mannopyranose.

**Table 2**
$^{1}H$ and $^{13}C$ chemical shifts for the extracellular polysaccharide GW-12.

| Sugar residues[a] | Chemical shifts (ppm)[b] | | | | | |
|---|---|---|---|---|---|---|
| | H1/C1 | H2/C2 | H3/C3 | H4/C4 | H5/C5 | H6/C6 |
| A | 5.08 | 4.11 | 3.88 | 3.71 | 3.93 | 3.83, 3.77 |
| | 103.8 | 74.83 | 73.1 | 68.5 | 72.2 | 62.5 |
| B | 5.16 | 4.06 | 4.00 | 3.74 | 3.93 | 3.84, 3.79 |
| | 99.8 | 80.0 | 72.2 | 71.7 | 72.8 | 67.0 |
| C | 5.26 | 4.15 | 3.99 | 3.74 | 3.99 | 3.74 |
| | 102.2 | 79.7 | 71.8 | 67.8 | – | 62.6 |
| D | 5.28 | 4.15 | 4.01 | 3.72 | 4.02 | 3.89, 3.89 |
| | 102.2 | 74.9 | 71.9 | 68.5 | – | 67.0 |

[a] A–D correspond to (1→)-α-D-Man*p*, (1→2,6)-α-D-Man*p*, (1→2)-α-D-Man*p* and (1→6)-α-D-Man*p*, respectively. Man*p*, mannopyranose.
[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 $^{13}C$.

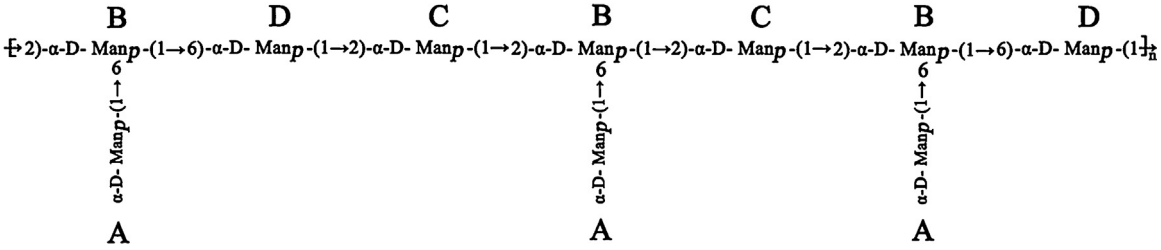


**Fig. 5.** One of the possible repeating structures of the extracellular polysaccharide GW-12 produced by the mangrove fungus *P. solitum* (Man*p*, mannopyranose; $n \approx 7$).

## 4. Conclusion

GW-12 excreted by the mangrove fungus *P. solitum* is a mannan with branches. Based on detailed one- and two-dimensional nuclear magnetic resonance spectroscopic analyses, the main chain of GW-12 is characterized to consist of (1→2)-α-D-mannopyranose and (1→6)-α-D-mannopyranose units, substituted at C-6 of (1→2)-α-D-mannopyranose residues by (1→)-linked α-D-mannopyranose residues. Further investigations on the extracellular polysaccharides from mangrove fungi are required.

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