Carbohydrate Polymers 93 (2013) 478–483

Contents lists available at SciVerse ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Structural elucidation of an extracellular polysaccharide produced by the marine fungus *Aspergillus versicolor*

Yin Chen[a,b], Wenjun Mao[a,*], Yan Gao[c], Xiancun Teng[a], Weiming Zhu[a], Yanli Chen[a], Chunqi Zhao[a], Na Li[a], Chunyan Wang[a], Mengxia Yan[a], Jimiao Shan[a], Cong Lin[a], Tao Guo[a]

[a] *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*
[b] *College of Food and Pharmacy, Zhejiang Ocean University, 18 Haiyuan Road, Zhoushan 316000, People's Republic of China*
[c] *National Oceanographic Center of Qingdao, 88 Xuzhou Road, Qingdao 266071, People's Republic of China*

ARTICLE INFO

Article history:
Received 18 September 2012
Received in revised form 6 December 2012
Accepted 7 December 2012
Available online 2 January 2013

Keywords:
Marine fungus
Aspergillus versicolor
Extracellular polysaccharide
FTIR
NMR
Structure

ABSTRACT

A homogenous extracellular polysaccharide, designated AWP, was isolated from the fermented liquid of the marine fungus *Aspergillus versicolor* from the coral *Cladiella* sp. and purified by anion-exchange and size-exclusion chromatography (SEC). Chemical and spectroscopic analyses, including one- and two-dimensional nuclear magnetic resonance (1D and 2D NMR) spectroscopy showed that AWP consisted of glucose and mannose in a molar ratio of 8.6:1.0, and its average molecular weight was estimated to be 500 kDa. AWP is a slightly branched extracellular polysaccharide. The backbone of AWP is mainly composed of (1→6)-linked α-D-glucopyranose residues, slightly branched by single α-D-mannopyranose units attached to the main chain at C-3 positions of the glucan backbone. The investigation demonstrated that AWP is a novel extracellular polysaccharide different from those of other marine microorganisms.

1. Introduction

Marine organisms, such as sponges, sea squirts, corals, worms and algae, host diverse and abundant symbiotic microorganisms (Olson & Kellogg, 2010). The microorganisms may be saprophytic, pathogenic, or may serve important positive functions in the ecosystem, and protect their hosts from pathogens (Armstrong, Yan, Boyd, Wright, & Burgess, 2001; Harder, Lau, Dobretsov, Fang, & Qian, 2003; Harel et al., 2008; Kramarsky-Winter et al., 2006; Rohwer, Breitbart, Jara, Azam, & Knowlton, 2002; Siboni et al., 2010). Recently, the interest in extracellular polysaccharides produced by symbiotic microorganisms from marine organisms is increasing because of the microorganisms are mostly new or inadequately described species (Miranda et al., 2008). The marine bacterium *Shewanella colwelliana* from *Crassostrea virginic* excreted a kind of acidic polysaccharide, which contained mannose, glucose, galactose and pyruvic acid (Sledjeski & Weiner, 1993). *Aeromonas* sp. HYD154 from a deep-sea hydrothermal vent polychaete annelid produced an acidic polysaccharide, with glucose, galactose, glucuronic acid, galacturonic acid and 4,6-pyruvic acidic galactose (Cambon-Bonavita, Raguenes, Jean, Vincent, & Guezennec, 2002).

The coral-associated fungus *Aspergillus versicolor* LCJ-5-4 could produce a mannoglucan, with the backbone of (1→6)-linked α-D-glucopyranose and (1→2)-linked α-D-mannopyranose units (Chen et al., 2012). The extracellular polysaccharides produced by symbiotic microorganisms from marine organisms represent a potential source to be explored (Dobretsov & Qian, 2004). In the present work, a novel extracellular polysaccharide was obtained from the culture liquids of the marine fungus *A. versicolor* from the coral *Cladiella* sp., and its structure was investigated by a combination of chemical, chromatographic (SEC, HPGPC, GC–MS) and spectroscopic (FTIR, 1D and 2D NMR) methods.

2. Methods

2.1. Materials

Dextran standards (M_w: 788, 404, 112, 47.3, 22.8, 11.8 and 5.9 kDa) were from Showa Denko K.K. (Tokyo, Japan). D-Glucose, D-mannose, D-galactose, L-rhamnose, D-xylose, L-fucose and D-glucuronic acid were from Sigma–Aldrich (St. Louis, MO, USA). Dialysis membranes (flat width 44 mm, molecular weight cut off 3500) were from Lvniao (Yantai, China). Q Sepharose Fast Flow and Sephacryl S-400/HR were from Amersham Biosciences (Uppsala, Sweden).

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

0144-8617/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.carbpol.2012.12.047

2.2. Microbial strains and culture conditions

A. versicolor was isolated from the coral *Cladiella* sp. collected from Hainan, China. It was identified according to its morphological characteristics and 18S rRNA sequences. Briefly, the fungus was cultivated in a liquid medium containing sorbitol (20 g/L), maltose (20 g/L), monosodium glutamate (10 g/L), KH_2PO_4 (0.5 g/L), $MgSO_4{\cdot}7H_2O$ (0.3 g/L), tryptophane (0.5 g/L), yeast extract (3 g/L), sea salt (33.3 g/L), pH 6.5 at 20 °C for 30 days, and 90 L of fermented broth were collected (Zhuang et al., 2011).

2.3. Isolation and purification of the extracellular polysaccharide

The fermented broth was filtered through cheesecloth to separate into fermentation liquids and mycelium. The fermentation liquids from *A. versicolor* cultures were concentrated, and three volumes of 95% (v/v) ethanol were added. The resulting precipitate was recovered by using centrifugation at 8000 rpm 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 by the method of Sevag (Matthaei, Jone, Martin, & Nirenberg, 1962). The crude polysaccharide was fractionated on a Q Sepharose Fast Flow column (300 × 30 mm) coupled to an AKTA FPLC system, eluting with 0, 0.3 and 2.5 M NaCl (Chen et al., 2012). The total sugar content of the fractions was determined by the phenol–sulfuric acid method. The fractions eluted with distilled water were pooled, and further purified on a Sephacryl S-400 column (100 cm × 3 cm) with 0.2 M NH_4HCO_3 as eluent. The major polysaccharide fraction was gathered and freeze-dried.

2.4. Analytical techniques

Total sugar content was measured by the phenol–sulfuric acid method using glucose as the standard (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956). Protein content was assayed according to the modified Lowry method (Bensadoun & Weinstein, 1976). Sulfate content was measured according to Silvestri, Hurst, Simpson, and Settine (1982). Uronic acid content was determined by the carbazole–sulfuric acid method using glucuronic acid as standard (Blumenkrantz & Asboe-Hansen, 1973). The homogeneity and molecular weight was determined by high performance gel permeation chromatography (HPGPC) on a Shodex OHpak SB-804 HQ column, and the column calibration was performed with dextran standards (Li et al., 2012). The monosaccharide compositions were measured by gas chromatography (GC) (Chen et al., 2011). Sugar identification was done by comparison with reference sugars (D-glucose, D-mannose, D-galactose, L-rhamnose, D-xylose and L-fucose), and inositol was added as the internal standard. Calculation of the molar ratio of the monosaccharide was carried out on the basis of the peak area of the monosaccharide.

2.5. Methylation analysis

Methylation analysis was performed according to the modified Hakomori method (Ciucanu & Kerek, 1984). Briefly, the polysaccharide in dimethyl sulfoxide was methylated using NaH and iodomethane. The completion of methylation was confirmed by FTIR spectroscopy as the disappearance of OH bands. After hydrolysis with 2 M 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, dissolved again in 100 μL dichloromethane, and then analyzed by gas chromatography–mass spectrometry (GC–MS) on a HP6890II/5973 instrument (Agilent Technologies Co. Ltd., USA) using a DB 225 fused silica capillary column (0.25 mm × 30 m). Identification of partially methylated alditol acetates was carried out on the basis of the retention time (t_R) and its mass fragmentation patterns (Chen et al., 2012; Hung et al., 2012).

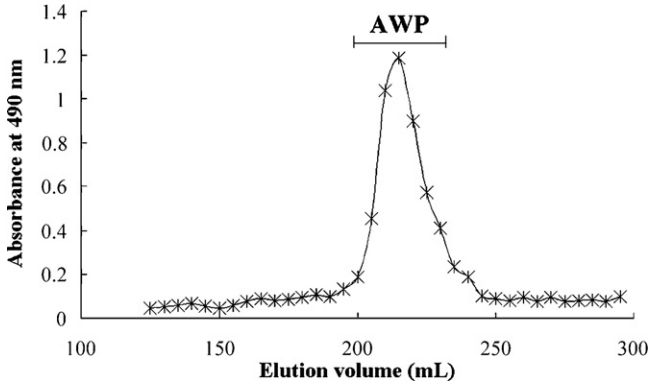

Fig. 1. Purification of the extracellular polysaccharide AWP from the marine fungus *A. versicolor* on a Sephacryl S-400 column.

2.6. IR spectroscopy analysis

For FTIR spectroscopy, the polysaccharide was mixed with KBr powder, ground and then pressed into a 1 mm pellets for FTIR measurement in the frequency range of 4000–500 cm^{-1} with the resolution of 4.0 cm^{-1} and 320 scans co-addition. FTIR spectrum of the polysaccharide was measured on a Nicolet Nexus 470 spectrometer by using the Nicolet Omnic software.

2.7. NMR spectroscopy analysis

Seventy mg of polysaccharide was deuterium exchanged by two successive freeze-drying steps in 99% D_2O (Sigma–Aldrich, Canada) 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 by using Delta NMR processing and control software. ^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. Chemical shifts are expressed in ppm using acetone as internal standard at 2.225 ppm for ^1H and 31.07 ppm for ^{13}C.

3. Results and discussion

3.1. Purification and chemical composition of the extracellular polysaccharide AWP

The two crude extracellular polysaccharides from the fermented broth of coral-associated fungus *A. versicolor* were isolated on a Q Sepharose Fast Flow column, and the polysaccharide fraction eluted with 0.3 M NaCl was reported (Chen et al., 2012). Here, the polysaccharide fraction eluted with distilled water was further purified by a Sephacryl S-400 column (Fig. 1), and an extracellular polysaccharide AWP was obtained. The yield of AWP from crude extracellular polysaccharide (0.24 g/L) was about 25%. As shown in Fig. 2, AWP appeared as a single peak in the HPGPC chromatogram, and its average molecular weight was about 500 kDa. AWP did not contain any sulfate esters and protein, and its uronic acid content was below the detection limit. GC analysis demonstrated that AWP was composed of glucose and mannose in a molar ratio of 8.6:1.0. It is noted that the chemical composition of AWP was different from that of

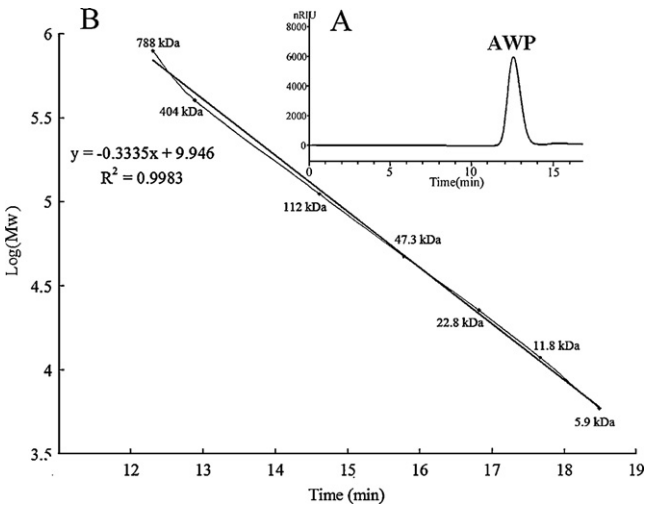

Fig. 2. (A) HPGPC chromatogram of AWP on a Shodex Ohpak SB-804 column and (B) calibration curve using molecular weight standards.

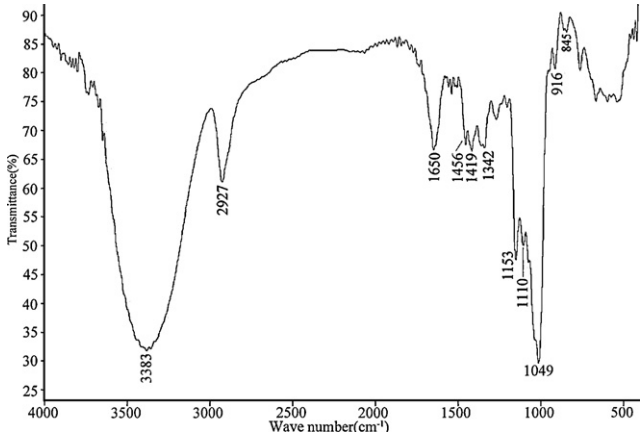

Fig. 3. IR spectrum of AWP.

the extracellular polysaccharide AVP isolated from coral-associated fungus *A. versicolor* (Chen et al., 2012).

3.2. Methylation analysis

The methylation analysis may offer valuable information about the position of the glycosidic linkage (Vieira, Mulloy, & Mourao, 1991). The identification and the proportions of the methylated alditol acetates of AWP are listed in Table 1. Large amounts of 1,5,6-tri-O-acetyl-2,3,4-tri-O-methyl-glucitol and small amounts of 1,3,5,6-tetra-O-acetyl-2,4-di-O-methyl-glucitol and 1,5-di-O-acetyl-2,3,4,6-tetra-O-methyl-mannitol were detected in AWP, suggesting that AWP was mainly composed of (1→6)-linked glucopyranose with small amounts of (1→3,6)-linked glucopyranose and (1→) mannopyranose residues. AWP could be recognized as a slightly branched polysaccharide with a (1→6)-linked glucopyranose backbone, like a polysaccharide dextran main chain (Naessens, Cerdobbel, Soetaert, & Vandamme, 2005), and the mannopyranose side chains attached to C-3 position of (1→6)-linked glucopyranose residues. The molar ratios were in good agreement with the monosaccharide composition of AWP obtained from GC analysis. The linkage pattern of the polysaccharide AWP was different from those of other extracellular polysaccharides from marine microorganisms (Chen et al., 2012; Guo et al., 2010).

3.3. IR spectrum analysis

IR spectrum of AWP was presented in Fig. 3. The broad and intense band at 3383 cm^{-1} was the result of valent vibrations OH groups and valent vibration of H_2O constitutional molecules (Mitić, Nikolić, Cakić, Premović, & Ilić, 2009), and the band at 2927 cm^{-1} was attributed to the stretching vibration of C—H bond (Chen et al., 2011; Mitić et al., 2011). The band at 1650 cm^{-1} was due to the bending vibrations of HOH, and the band at 1419 cm^{-1} originated from the bending vibrations of O—H bond (Cakić, Mitić, Nikolić, Ilić, & Nikolić, 2008; Mitić, Cakić, & Nikolić, 2010; Mitić et al., 2009, 2011). The bands at 1456 and 1342 cm^{-1} were the absorption peaks of variable angle vibration of C—H bond. The band at 1153 cm^{-1} was assigned to valent vibrations of the C—O—C bond and glycosidic bridge. The band at 916 cm^{-1} was due to the asymmetric telescopic vibration of glucopyranose ring, and the band at 845 cm^{-1} indicated the presence of α-anomeric configuration. The bands at 916 and 845 cm^{-1} also indicated that the glucopyranose units in AWP had a C_1 chair conformation (Mitić et al., 2009, 2010; Shingel, 2002).

3.4. NMR spectroscopy analysis

In the ^{13}C NMR spectrum of AWP (Fig. 4A), six major signals among 98–65 ppm were attributed to C-1–C-6 of glucopyranose units. The chemical shift of C-6 was displaced to the low magnetic field at 66.7 ppm, which indicated that the glucopyranose was substituted at C-6 (Uzochukwu et al., 2002). It was deduced that the backbone of AWP was composed of →6)-α-D-Glc*p*-(1→ units. In the anomeric region of the ^1H NMR spectrum (Fig. 4B), the resonance at 5.02 ppm was due to the H-1 of the (1→6)-linked D-glucopyranose units (Purama, Goswami, Khan, & Goyal, 2009). The weak resonance peak at 5.36 ppm indicated the existence of a little branched linkage (Seymour, 1979), and it was ascribed to H-1 of the (1→3,6)-linked D-glucopyranose units. The anomeric proton signals at 5.02 and 5.36 ppm were assigned to α-configuration pyranose units.

The assignments for different resonances of 2D NMR experiments, including ^1H–^1H COSY, ^1H–^{13}C HMQC and ^1H–^{13}C HMBC were used to further assign the chemical shifts of the main spin system. ^1H NMR spin systems of the polysaccharide were assigned by ^1H–^1H COSY (Fig. 4C). Combined with the analysis of the ^1H–^{13}C HMQC (Fig. 4D), the assignment of all the signals of the major unit could be completed. The presence of strong cross peak H-1/C-6 and H-6/C-1 on ^1H–^{13}C HMBC (Fig. 4E) confirmed that (1→6)-α-D-Glc*p* was the main pattern of linkage. The correlation signals assignment of ^1H–^1H COSY, ^1H–^{13}C HMQC and ^1H–^{13}C HMBC further confirmed the conclusion (Maina, Tenkanen, Maaheimo, Juvonen, & Virkki, 2008; Purama et al., 2009; Yan, Li, Wang, & Wu, 2010). The ^1H and ^{13}C NMR chemical shifts are summarized in Table 2.

Table 1
Results from methylation analysis of AWP.

Methylated sugar[a]	Primary mass fragments (*m*/*z*)	Molar ratio	Deduced linkage type
2,3,4,6-$(Me)_4$-Man	87, 101, 117, 145, 161, 205	1.0	Man*p*-(1→
2,3,4-$(Me)_3$-Glc	87, 101, 117, 129, 161, 189, 233	7.7	→6)-Glc*p*-(1→
2,4-$(Me)_2$-Glc	87, 101, 117, 129, 189, 233, 305	1.1	→3,6)-Glc*p*-(1→

[a] 2,3,4,6-$(Me)_4$-Man, 2,3,4-$(Me)_3$-Glc and 2,4-$(Me)_2$-Glc correspond to 1,5-di-O-acetyl-2,3,4,6-tetra-O-methyl-D-mannitol, 1,5,6-tri-O-acetyl-2,3,4-tri-O-methyl-D-glucitol and 1,3,5,6-tetra-O-acetyl-2,4-di-O-methyl-D-glucitol, respectively.

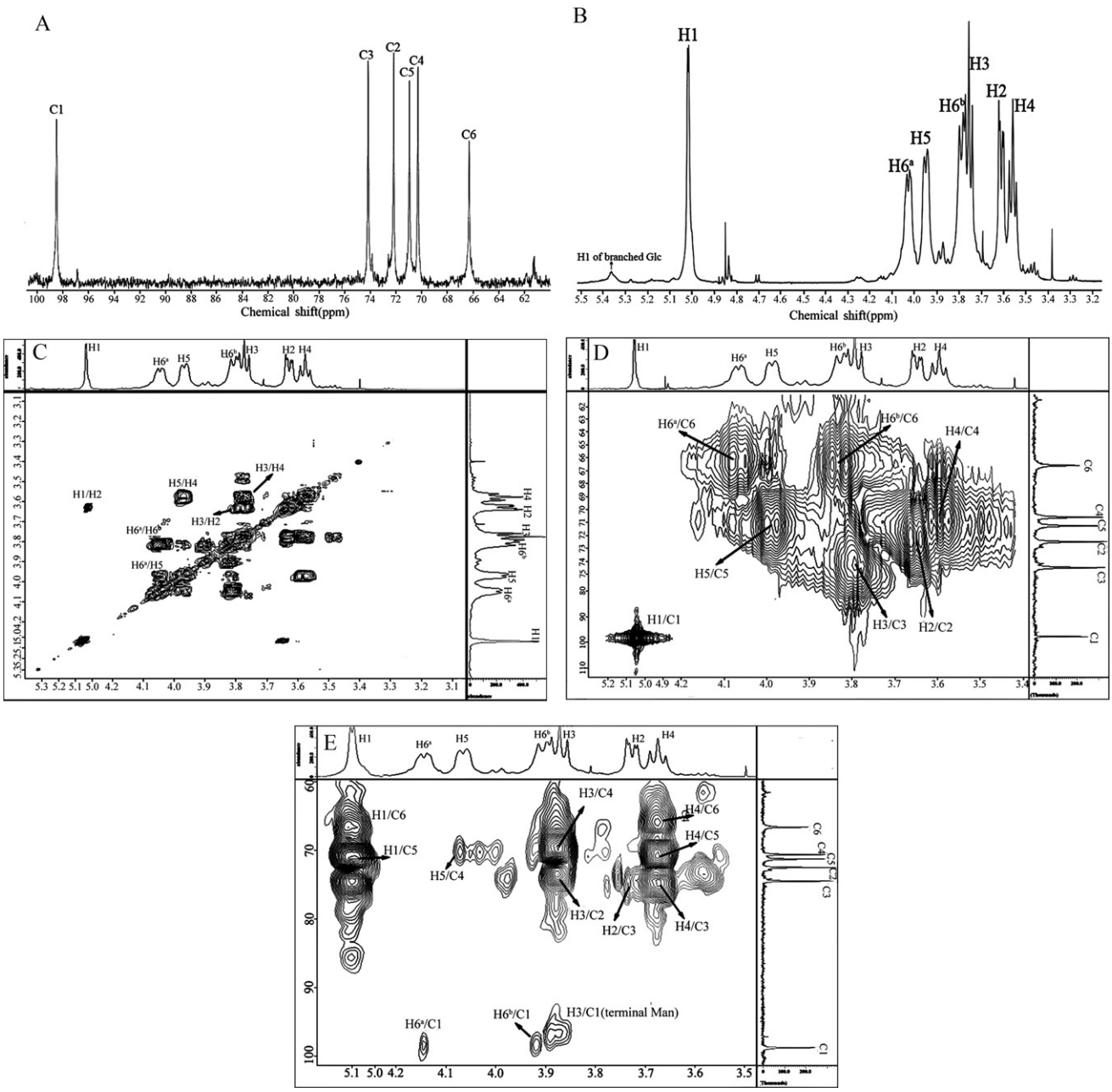

Fig. 4. NMR spectra of AWP. Spectra were performed at 23 °C on a JEOL ECP 600 MHz spectrometer using acetone as internal standard. (A) ^{13}C NMR and DEPT spectra, (B) ^{1}H NMR spectrum, (C) ^{1}H–^{1}H COSY spectrum, (D) ^{1}H–^{13}C HMQC spectrum, and (E) ^{1}H–^{13}C HMBC spectrum.

Because of the strong signals of (1→6)-linked α-D-glucopyranose units, other signals were relatively weak and overlapped. It is hard to assign these signals clearly. The weak resonance at 97.2 ppm could be attributed to the anomeric carbon of non-reducing terminal mannopyranose. The peak at 61.5 ppm was attributed to C-6 of the terminal mannopyranose. The correlation signals of ^{1}H–^{13}C HMBC at 3.59/97.2 ppm were presumed to be the correlation of C-1 of terminal mannopyranose and H-3 of

Table 2
^{1}H and ^{13}C chemical shifts for AWP.

Sugar residues[d]	^{1}H/^{13}C chemical shifts (ppm)[c]					
	H1/C1	H2/C2	H3/C3	H4/C4	H5/C5	H6[a], H6[b]/C6
→6)-α-D-Glc*p*-(1→	5.02/98.80	3.61/72.48	3.75/74.82	3.54/70.59	3.95/71.26	4.03,3.82/66.0
$^{3}J_{H,H}$ (Hz)	3.0	9.75	9.36	9.36	8.28	6.9

[a] Equatorial bond.
[b] Axial bond.
[c] 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.
[d] Glc*p*: glucopyranose.

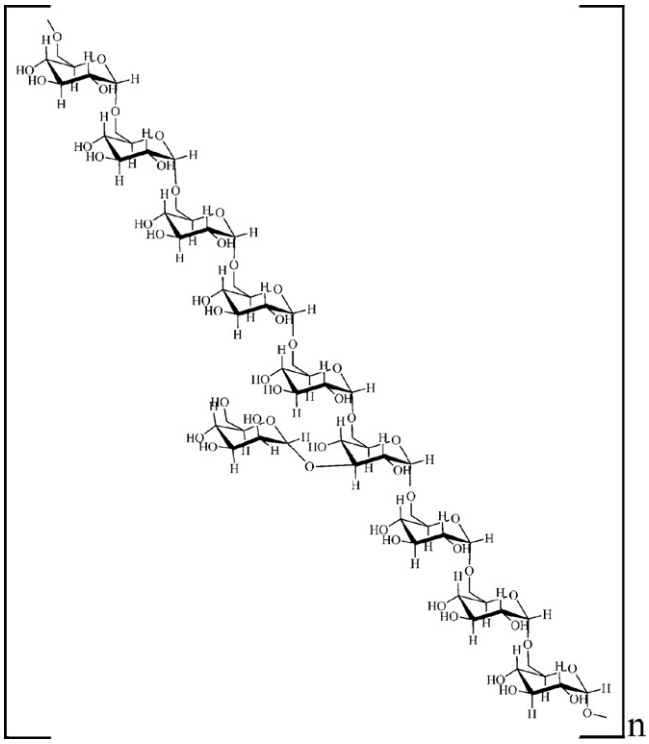

Fig. 5. Proposed structure of the extracellular polysaccharide AWP produced by *A. versicolor* ($n \approx 300$).

the (1→6)-linked D-glucopyranose. Thus, it was supposed that the mannopyranose was linked to C-3 position of the (1→6)-linked D-glucopyranose backbone.

All the results demonstrated that the backbone of AWP contained a large number of consecutive (1→6)-linked glucopyranose units with minor amount of branches. The branching was composed of single non-reducing-end mannopyranose units which were linked to C-3 position of (1→6)-linked D-glucopyranose units. On average, there was one branching point for every 9 units on the backbone. The proposed structure of AWP was presented in Fig. 5.

The extracellular polysaccharide AWP from coral-associated fungus *A. versicolor* had different structural characteristics from other extracellular polysaccharides from *Aspergillus* species (Gómez-Miranda et al., 2003; Tischer, Gorin, de Souza, & Barreto-Bergter, 2002). The polysaccharides from mycelia of *A. versicolor* were characterized to be phosphonogalactomannans, which contained a mannan core, substituted at *O*-2 and *O*-6 with side chains of (1→5)-linked β-galactofuranosyl units, phosphodiester groups were present principally as bridges between C-1 of units of α-mannopyranose and C-6 of those of 5-*O*-substituted β-galactofuranose (Tischer et al., 2002). The polysaccharide from *Aspergillus wentii* corresponded to the following repeating unit: [→3)-β-D-Gal*f*-(1→5)-β-D-Gal*f*-(1→]$_n$→mannan core. The mannan core is constituted by a (1→6)-α-mannan backbone, substituted at positions 2 by chains from 1 to 7 residues of (1→2) linked α-mannopyranoses (Gómez-Miranda et al., 2003). In addition, it is noted that the structure of AWP was different from that of the extracellular polysaccharide AVP isolated from the fermented broth of *A. versicolor* (Chen et al., 2012). The backbone of AVP was composed of (1→6)-linked α-D-glucopyranose and (1→2)-linked α-D-mannopyranose units, and the mannopyranose residues in the backbone were substituted mainly at C-6 by the side chain of (1→2)-linked α-D-mannopyranose trisaccharides units. Symbiotic microorganisms have close relationship with the hosts, and may be involved in organism biosynthesis, metabolism and other life activities of large hosts (Croft et al., 2005; Dobretsov & Qian, 2004). The present result suggested that symbiotic microorganisms from marine organisms could be a potential source of extracellular polysaccharides with unique structures, and be worth being further investigated.

4. Conclusion

AWP produced by the marine fungus *A. versicolor* from the coral *Cladiella* sp. is a novel extracellular polysaccharide with different structural characteristics from extracellular polysaccharides excreted by other marine microorganisms. The main chain of AWP consists of (1→6)-linked α-D-glucopyranose residues with minor amounts of substitutions at C-3 by (1→)-linked α-D-mannopyranose. Further studies are underway to characterize its physical and chemical properties.

Acknowledgements

This work was supported by Science and Technology Development Program of Shandong Province, China (2010GHY10509), National Oceanographic Center of Qingdao of China, National Natural Science Foundation of China (41076086) and Doctoral Program of Higher Education of China (20090132110015).

References

Armstrong, E., Yan, L. M., Boyd, K. G., Wright, P. C., & Burgess, J. G. (2001). The symbiotic role of marine microbes on living surfaces. *Hydrobiologia*, *461*, 37–40.

Bensadoun, A., & Weinstein, D. (1976). Assay of proteins in presence of interfering materials. *Analytical Chemistry*, *70*, 241–256.

Blumenkrantz, N., & Asboe-Hansen, G. (1973). New method for quantitative determination of uronic acids. *Analytical Biochemistry*, *54*, 484–489.

Cakić, M., Mitić, Ž., Nikolić, G. S., Ilić, L., & Nikolić, G. M. (2008). The investigations of bioactive copper (II) complexes with reduced low-molar dextran. *Spectroscopy*, *22*, 177–185.

Cambon-Bonavita, M. A., Raguenes, G., Jean, J., Vincent, P., & Guezennec, J. (2002). A novel polymer produced by a bacterium isolated from a deep-sea hydrothermal vent polychaete annelid. *Journal of Applied Microbiology*, *93*, 310–315.

Chen, Y., Mao, W. J., Tao, H. W., Zhu, W. M., Qi, X. H., Chen, Y. L., et al. (2011). Structural characterization and antioxidant properties of an exopolysaccharide produced by the mangrove endophytic fungus *Aspergillus* sp. Y16. *Bioresource Technology*, *102*, 8179–8184.

Chen, Y., Mao, W. J., Yang, Y. P., Teng, X. C., Zhu, W. M., Li, H. Y., et al. (2012). Structure and antioxidant activity of an extracellular polysaccharide from coral-associated fungus, *Aspergillus versicolor* LCJ-5-4. *Carbohydrate Polymers*, *87*, 218–226.

Ciucanu, I., & Kerek, F. (1984). A simple and rapid method for the permethylation of carbohydrates. *Carbohydrate Research*, *131*, 209–217.

Croft, M. T., Lawrence, A. D., Raux-Deery, E., Martin, J., Warren, M. J., & Smith, A. G. (2005). Algae acquire vitamin B12 through a symbiotic relationship with bacteria. *Nature*, *383*, 90–93.

Dobretsov, S., & Qian, P. Y. (2004). The role of epibotic bacteria from the surface of the soft coral *Dendronephthya* sp. in the inhibition of larval settlement. *Journal of Experimental Marine Biology and Ecology*, *299*, 35–50.

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*, 350–356.

Gómez-Miranda, B., Prieto, A., Leal, J. A., Ahrazem, O., Jiménez-Barbero, J., & Bernabé, M. (2003). Differences among the cell wall galactomannans from *Aspergillus wentii* and *Chaetosartorya chrysella* and that of *Aspergillus fumigatus*. *Glycoconjugate Journal*, *20*, 239–246.

Guo, S. D., Mao, W. J., Han, Y., Zhang, X. H., Yang, C. L., Chen, Y., et al. (2010). Structural characteristics and antioxidant activities of the extracellular polysaccharides produced by marine bacterium *Edwardsiella tarda*. *Bioresource Technology*, *101*, 4729–4732.

Harder, T., Lau, S. C. K., Dobretsov, S., Fang, T. K., & Qian, P. Y. (2003). A distinctive epibiotic bacterial community on the soft coral *Dendronephthya* sp. and antibacterial activity of coral tissue extracts suggest chemical mechanism against bacterial epibiosis. *FEMS Microbiology Ecology*, *43*, 337–347.

Harel, M., Ben-Dov, E., Rasoulouniriana, D., Siboni, N., Kramarsky-Winter, E., Loya, Y., et al. (2008). A new Thraustochytrid, strain *Fng1*, isolated from the surface mucus of the hermatypic coral *Fungia granulosa*. *FEMS Microbiology Ecology*, *64*, 378–387.

Hung, W. T., Wang, S. H., Chen, Y. T., Yu, H. M., Chen, C. H., & Yang, W. B. (2012). MALDI-TOF MS analysis of native and permethylated or benzimidazole-derivatized polysaccharides. *Molecules*, *17*, 4950–4961.

Kramarsky-Winter, E., Harel, M., Siboni, N., Ben-Dov, E., Brickner, I., Loya, Y., et al. (2006). Identification of a protist–coral association and its possible ecological role. *Marine Ecology Progress Series*, *317*, 67–73.

Li, H. Y., Mao, W. J., Hou, Y. J., Gao, Y., Qi, X. H., Zhao, C. Q., et al. (2012). Preparation, structure and anticoagulant activity of a low molecular weight fraction produced by mild acid hydrolysis of sulfated rhamnan from *Monostroma latissimum*. *Bioresource Technology*, *114*, 414–418.

Maina, N. H., Tenkanen, M., Maaheimo, H., Juvonen, R., & Virkki, L. (2008). NMR spectroscopic analysis of exopolysaccharides produced by *Leuconostoc citreum* and *Weissella confusa*. *Carbohydrate Research*, *343*, 1446–1455.

Matthaei, J. H., Jone, O. W., Martin, R. G., & Nirenberg, M. W. (1962). Characteristics and composition of RNA coding units. *Proceedings of the National Academy of Sciences*, *48*, 666–667.

Miranda, C. C. B. O., Dekker, R. F. H., Serpeloni, J. M., Fonseca, E. A. L., Cólus, I. M. S., & Barbosa, A. M. (2008). Anticlastogenic activity exhibited by botryosphaeran, a new exopolysaccharide produced by *Botryosphaeria rhodina* MAMB-05. *International Journal of Biological Macromolecules*, *42*, 172–177.

Mitić, Ž., Cakić, M., & Nikolić, G. (2010). Fourier-transform IR spectroscopic investigations of Cobalt (II)–dextran complexes by using D_2O isotopic exchange. *Spectroscopy*, *24*, 269–275.

Mitić, Ž., Cakić, M., Nikolić, G. M., Nikolić, R., Nikolić, G. S., Pavlović, R., et al. (2011). Synthesis, physicochemical and spectroscopic characterization of copper (II)–polysaccharide pullulan complexes by UV–vis, ATR-FTIR, and EPR. *Carbohydrate Research*, *346*, 434–441.

Mitić, Ž., Nikolić, G. S., Cakić, M., Premović, P., & Ilić, L. (2009). FTIR spectroscopic characterization of Cu (II) coordination compounds with exopolysaccharide pullulan and its derivatives. *Journal of Molecular Structure*, *924–926*, 264–273.

Naessens, M., Cerdobbel, A., Soetaert, W., & Vandamme, E. J. (2005). Leuconostoc dextransucrase and dextran: Production, properties and applications. *Journal of Chemical Technology and Biotechnology*, *80*, 845–860.

Olson, J. B., & Kellogg, C. A. (2010). Microbial ecology of corals, sponges, and algae inmesophotic coral environments. *FEMS Microbiology Ecology*, *73*, 17–30.

Purama, R. K., Goswami, P., Khan, A. T., & Goyal, A. (2009). Structural analysis and properties of dextran produced by *Leuconostoc mesenteroides* NRRL B-640. *Carbohydrate Polymers*, *76*, 30–35.

Rohwer, F., Breitbart, M., Jara, J., Azam, F., & Knowlton, N. (2002). Diversity and distribution of coral-associated bacteria. *Marine Ecology Progress Series*, *243*, 1–10.

Seymour, F. R. (1979). Correlation of the structure of dextran to their ^{1}H NMR spectra. *Carbohydrate Research*, *74*, 77–92.

Shingel, K. I. (2002). Determination of structural peculiarities of dextran, pullulan and γ-irradiated pullulan by Fourier-transform IR spectroscopy. *Carbohydrate Research*, *337*, 1445–1451.

Sledjeski, D. D., & Weiner, R. M. (1993). Production and characterization of monoclonal-antibodies specific for *Shewanella colwelliana* exopolysaccharide. *Applied and Environmental Microbiology*, *59*, 1565–1572.

Siboni, N., Rasoulouniriana, D., Ben-Dov, E., Kramarsky-Winter, E., Sivan, A., Loya, Y., et al. (2010). Stramenopile microorganisms associated with the massive coral *Favia* sp. *Journal of Eukaryotic Microbiology*, *57*, 236–244.

Silvestri, L. J., Hurst, R. E., Simpson, L., & Settine, J. M. (1982). Analysis of sulfate in complex carbohydrates. *Analytical Biochemistry*, *123*, 303–309.

Tischer, C. A., Gorin, P. A. J., de Souza, M. B., & Barreto-Bergter, E. (2002). Structures of phosphonogalactomannans isolated from mycelia of *Aspergillus versicolor*. *Carbohydrate Polymers*, *49*, 225–230.

Uzochukwu, S., Balogh, E., Loefler, R. T., & Ngoddy, P. O. (2002). Structural analysis by ^{13}C nuclear magnetic resonance spectroscopy of glucan extracted from natural palm wine. *Food Chemistry*, *76*, 287–291.

Vieira, R. P., Mulloy, B., & Mourao, P. A. S. (1991). Structure of a fucose-branched chondroitin sulfate from sea cucumber, Evidence for the presence of 3-O-sulfo-beta-D-glucuronosyl residues. *The Journal of Biological Chemistry*, *266*, 13530–13536.

Yan, J. K., Li, L., Wang, Z. M., & Wu, J. Y. (2010). Structural elucidation of an exopolysaccharide from mycelial fermentation of a *Tolypocladium* sp. fungus isolated from wild *Cordyceps sinensis*. *Carbohydrate Polymers*, *79*, 125–130.

Zhuang, Y. B., Teng, X. C., Wang, Y., Liu, P. P., Li, G. Q., & Zhu, W. M. (2011). New quinazolinone alkaloids within rare amino acid residue from coral-associated fungus, *Aspergillus versicolor* LCJ-5-4. *Organic Letters*, *13*, 1130–1133.