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Characterization of polysaccharides from *Ganoderma* spp. using saccharide mapping

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ABSTRACT

Polysaccharides from *Ganoderma* spp. and their adulterants were firstly investigated and compared using saccharide mapping, enzymatic (endo-1,3-β-D-glucanase and pectinase) digestion followed by polysaccharide analysis using carbohydrate gel electrophoresis analysis. The results showed that both 1,3-β-D-glucosidic and 1,4-α-D-galactosiduronic linkages were existed in Lingzhi (*Ganoderma lucidum* and *Ganoderma sinense*), and the similarity of polysaccharides from *G. lucidum* and *G. sinense* was high, which may contribute to rational use of Lingzhi. Different species of *Ganoderma* and their adulterants can be differentiated based on the saccharide mapping, which is helpful to well understand the structural characters of polysaccharides from different species of *Ganoderma* and to improve the quality control of polysaccharides in Lingzhi.

1. Introduction

Ganoderma, known as "Lingzhi" in Chinese or "Reishi" in Japanese, is a basidiomycete white rot fungus which has been used for medicinal purpose for centuries. So far, 98 species of *Ganoderma* have been found in China, but only two species, *Ganoderma lucidum* and *Ganoderma sinense*, are officially recorded as Lingzhi in China. Sure, Lingzhi has been used for prevention and treatment of a variety of diseases (Paterson, 2006; Xu, Chen, Zhong, Chen, & Wang, 2011). Triterpenes (Boh, Berovic, Zhang, & Lin, 2007; Paterson, 2006) and polysaccharides (Fan, Li, Deng, & Ai, 2012; Han, Chan, Yu, et al., 2012; Han, Chan, Dong, et al., 2012; Meng et al., 2011; Paterson, 2006; Xu, Chen, et al., 2011; Zhao, Dong, Chen, & Hu, 2010) are usually considered as main bioactive components of Lingzhi. Unfortunately, the content of triterpenes is greatly variant among different species of *Ganoderma* (Su, Yang, Ho, Hu, & Sheu, 2001; Wang et al., 2006; Zhao et al., 2006). Though polysaccharides were used as the marker for quality control of Lingzhi (Chinese Pharmacopoeia Commission, 2010), only few works have been performed for the comparison of polysaccharides in *G. lucidum* and *G. sinense* (Di, Chan, Leung, & Huie, 2003; Guan & Li, 2010; Xie et al., 2012) due to the structural complicity of polysaccharides. Therefore, comparison of polysaccharides from different species of *Ganoderma* is very important for quality control of *Ganoderma*. Indeed, "saccharide mapping", established in our laboratory for qualitative analysis of polysaccharides from different traditional Chinese medicines (Guan & Li, 2010), has been used for comparison of polysaccharides from *G. lucidum* and *G. sinense* based on acidic hydrolysis followed by HPTLC, enzymatic digestion and HPSEC analysis (Guan, Zhao, Feng, Hu, & Li, 2011; Xie et al., 2012; Xu, Guan, Chen, Zhao, & Li, 2011). However, HPTLC is usually not suitable for analysis of saccharides with high degree of polymerization (DP), while single column HPSEC is difficult to simultaneously separate both polysaccharides and their hydrolysates. In addition, the sensitivity of ELSD and RID, which are widely used for detection of saccharides without UV absorbance, is poor. Therefore, a sensitive, good resolution and high throughput method is necessary for saccharide mapping, analysis of enzymatic hydrolysates of polysaccharides.

Polysaccharide analysis using carbohydrate gel electrophoresis (PACE) is a sensitive and simple method for studying polysaccharide characters, which relies on derivatization of the reducing ends of sugars with a fluorophore and followed by electrophoresis under optimized conditions in polyacrylamide gels (Goubet, Dupree, & Johansen, 2011). In this study, polysaccharides from *Ganoderma* spp. and some adulterants were firstly analyzed and compared using partial acid hydrolysis and enzymatic hydrolysis,

Abbreviations: ANTS, 8-aminonaphthalene-1,3,6-trisulfonic acid; DP, degree of polymerization; DMSO, dimethyl sulfoxide; ELSD, evaporative light scattering detector; HPTLC, high-performance thin-layer chromatography; HPSEC, high-performance size-exclusion chromatography; PACE, carbohydrate analysis by carbohydrate gel electrophoresis; RID, refractive index detection; SMC-GL, simulative mean chromatogram of *Ganoderma. lucidum*; SMC-GS, simulative mean chromatogram of *Ganoderma. sinense*; TFA, trifluoroacetic acid.

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Table 1
Summary of the investigated samples.

No.	Code	Species	Source
1	GL01	*Ganoderma lucidum*	Anhui Province
2	GL02	*G. lucidum*	Anhui Province
3	GL03	*G. lucidum*	Anhui Province
4	GL04	*G. lucidum*	Shandong Province
5	GL05	*G. lucidum*	Shandong Province
6	GL06	*G. lucidum*	Shandong Province
7	GL07	*G. lucidum*	Shandong Province
8	GL08	*G. lucidum*	Sichuan Province
9	GL09	*G. lucidum*	Hunan Province
10	GL10	*G. lucidum*	Zhejiang Province
11	GS00	*Ganoderma sinense*	Anhui Province
12	GS01	*G. sinense*	Anhui Province
13	GS02	*G. sinense*	Anhui Province
14	GS03	*G. sinense*	Anhui Province
15	GS04	*G. sinense*	Anhui Province
16	GS05	*G. sinense*	Shandong Province
17	GS06	*G. sinense*	Shandong Province
18	GS07	*G. sinense*	Guizhou Province
19	GS08	*G. sinense*	Hunan Province
20	GS09	*G. sinense*	Sichuan Province
21	GS10	*G. sinense*	Guangxi Province
22	GT	*Ganoderma tropicum*	Macao
23	GTC	*G. tropicum*	Lab cultured
24	GA	*Ganoderma applanatum*	Commercial
25	GC	*Ganoderma capense*	Commercial
26	GSM	*Ganoderma sessile*	Commercial
27	FF	*Fomes fomentarius*	Commercial
28	LR	*Lignosus rhinoceros*	Commercial
29	TV	*Trametes versicolor*	Commercial
30	IO	*Inonotus obliquus*	Commercial
31	AM	*Amauroderma* spp.	Commercial

respectively, followed by PACE analysis. The results were helpful to well understand the structural characters of polysaccharides from different species of *Ganoderma* and to ensure rational use of Lingzhi.

2. Materials and methods

2.1. Materials and chemicals

Ten batches of dried fruiting bodies of *G. lucidum* (GL) and eleven batches of dried fruiting bodies of *G. sinense* (GS) were collected from different places of China. The dried fruiting bodies of commercial adulterants, including *Ganoderma applanatum*, *Ganoderma capense*, *Ganoderma sessile*, *Fomes fomentarius*, *Trametes versicolor*, *Inonotus obliquus*, *Lignosus rhinoceros* and *Amauroderma* spp. were purchased from Rui Zhi Tang Corporation (Table 1). The dried fruiting body of *Ganoderma tropicum* was collected from Macao. Identities of these samples were confirmed by Professor Xiaolan Mao, Institute of Microbiology, Chinese Academy of Sciences. The dried fruiting body of cultured *G. tropicum* was produced in our laboratory. The voucher specimens (Fig. 1) were deposited at the Institute of Chinese Medical Sciences, University of Macau, Macao, China.

D-Glucose, D-lactose, polygalacturonan and dextran 2000 were purchased from Sigma (St. Louis, MO, USA). Pectinase (endopolygalacturonase, EC 3.2.1.15), dextranase (EC 3.2.1.11) and 1,3-β-D-glucanase (endo-1,3-β-D-glucanase, EC 3.2.1.39) were purchased from Megazyme (Wicklow, Ireland). ANTS (8-aminonaphthalene-1,3,6 trisulphonic acid) was purchased from Tokyo Chemical Industry (Tokyo, Japan). Polyacrylamide containing a ratio of acrylamide/N,N-methylenebisacrylamide (19:1) was obtained from Bio-Rad (Hercules, CA, USA). Deionized water was prepared by a Millipore Milli-Q Plus system (Millipore, Bedford, MA, USA). All the other reagents were of analytical grade.

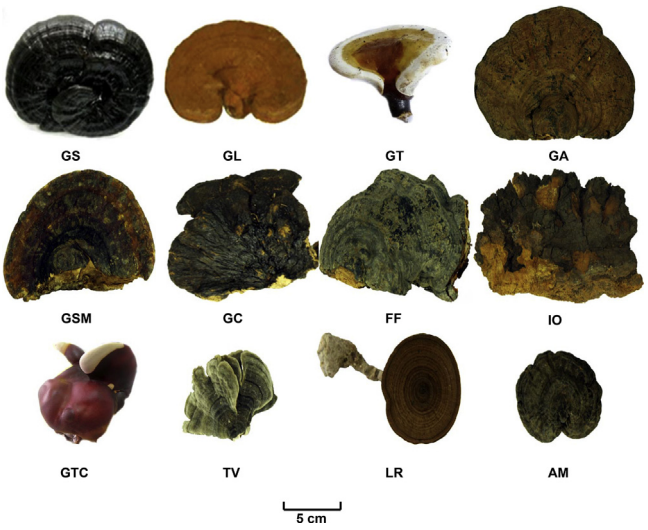

Fig. 1. The typical materials of *Ganoderma* and the adulterants. The sample codes were the same as in Table 1.

2.2. Preparation of polysaccharides

The samples were carefully cleaned and cut into slices, dried at 40 °C for 24 h, and pulverized. The powders of sample materials (2.0 g) were suspended in 40 mL of deionized water and extracted with microwave-assisted extraction (Multiwave 3000, Anton paar GmbH, Graz, Austria). The microwave irradiation program was performed at 500 W and 95 °C for 15 min. After microwave heating, the reactor was immediately cooled for 15 min. Then the extracted liquor was centrifuged at 4000 × *g* for 10 min (Allegre X-15R centrifuge, Beckman Coulter, Fullerton, CA, USA). An aliquot of supernatant (40 mL) was evaporated to 10 mL of solution under vacuum. Then ethanol (95%, w/v) was added to final concentration of 80% (v/v) for precipitation of crude polysaccharides. After standing for 12 h at 4 °C, centrifugation (4500 × *g* for 15 min) was performed. The precipitate was dried on water bath (80 °C), and then redissolved in 5 mL of hot water (80 °C). After centrifugation (4500 × *g* for 15 min), the supernatant was transferred to an ultracentrifugal filter (molecular weight cutoff: 10 kDa, Millipore, Billerica, MA, USA), and then the low molecular weight compounds (Mw < 10 kDa) were removed by centrifugation (4500 × *g*, 20 min, 25 °C). The content of sugar in crude polysaccharides was determined using phenol–sulfuric acid assay with glucose as reference standard (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956; Masuko et al., 2005). Finally, the crude polysaccharides, which were prepared in duplicates, were obtained for further analysis.

2.3. Partial acid hydrolysis of polysaccharides

The crude polysaccharides (5 mg/mL, 100 μL) were treated with TFA at a final concentration of 0.5 mol/L at a total volume of 250 μL. The suspensions were incubated at 80 °C for 5 h. After hydrolysis, the hydrolysates were washed with methanol and evaporated to dryness with a nitrogen evaporator at 40 °C for three times to remove the residue of TFA. The dried products were stored in 4 °C before derivatization with ANTS. The polysaccharide standard (dextran) was treated with TFA under the same conditions, and the hydrolysates were used for optimization of PACE.

2.4. Enzymatic digestion of polysaccharides

Polysaccharides solutions (5 mg/mL, 100 μL) were mixed with certain enzyme (the final concentration of endo-1,3-β-D-glucanase and pectinase were 2.5 U/mL and 10 U/mL, respectively) in a total volume of 1000 μL and digested overnight (12 h) at 40 °C. Then the mixtures were heated at 80 °C for 30 min to stop the enzymatic digestion. After centrifugation (10,000 × *g*) at room temperature for 20 min (CT15RE, Hitachi Koki Co., Ltd., Tokyo, Japan), the supernatants were evaporated to dryness with a nitrogen evaporator at 40 °C and then were used for derivatization. Polysaccharide solution without enzymes, treated as described above, was used as blank control. The polysaccharides standards, polygalacturonan and dextran (5 mg/mL, 100 μL), were treated with pectinase and dextranase, respectively, under the above conditions. The enzymatic hydrolysates and bromophenol blue were used as markers for PACE.

2.5. Derivatization with 8-aminonaphthalene-1,3,6-trisulfonic acid (ANTS)

The derivatization was carried out according to the reported method with minor modification (Goubet, Jackson, Deery, & Dupree, 2002). Briefly, ANTS was prepared in acetic acid/water (3:17, v/v) at a final concentration of 0.1 mol/L. $NaCNBH_3$ (1 mol/L) was solubilized in DMSO for ANTS derivatization. To each dry sample, 50 μL of ANTS solution and 50 μL of $NaCNBH_3$ solution were added. The reagents were mixed, centrifuged and incubated at 37 °C for 17 h. Then the solution was evaporated to dryness with a nitrogen evaporator at 40 °C, and the derivatized sugars were resuspended in 1 mL of urea (6 mol/L) solution and stored at −20 °C before use.

2.6. Electrophoresis

All samples (1–5 μL depending of the sugar concentration) were separated using a vertical slab gel electrophoresis apparatus, Mini-Protean Tetra System (Bio-Rad, Hercules, CA, USA), with 10 cm plates, 1.0-mm spacer, and well of width 0.3 cm or 0.5 cm. Electrophoresis was performed at 0 °C to reduce Joule heating. Electrophoresis of 30% (w/v) polyacrylamide in the resolving gel with a stacking gel of 8% (w/v) polyacrylamide was used for separation of partial acid and enzymatic hydrolysates. Meanwhile, both resolving and stacking gels were prepared in 0.1 mol/L Tris–boric acid (pH 8.2). The gels were cast and cooled at least 2 h before use in order to allow complete polymerization of acrylamide. The electrophoresis buffer (cooled at 0 °C) was 0.1 mol/L Tris adjusted to pH 8.2 with boric acid. For optimization of electrophoresis conditions, the effects of voltage (800 V, 700 V, 600 V, 500 V and 300 V) on gel resolution were investigated. The samples were electrophoresed first at 200 V for 20 min and then at 800 V, 700 V, 600 V, 500 V and 300 V for 50 min, 80 min, 120 min, 180 min and 270 min, respectively, to move bromophenol blue (migration indicator) to the designed level. All runs were performed at least two times independently.

2.7. Method validation

The method repeatability and sample stability were investigated. In brief, dextran ladders derived from the partial acid hydrolysates of dextran were prepared thrice and electrophoresed twice independently under the optimal conditions. Moreover, the enzymatic hydrolysates of polysaccharides from samples GL01–GL05 were prepared and electrophoresed twice independently according to the method mentioned above. For investigation of the sample stability, the dextran ladders and enzymatic hydrolysate of polysaccharides from sample GS09 were prepared, and stored at −20 °C for electrophoresis on successive day 1, 3 and 5.

2.8. Gels imaging and data analysis

Gels were imaged using an InGenius LHR CCD camera system (Syngene, Cambridge, UK) under UV 365 nm. The optical densities of bands in electronic images and digital scanning profiles were generated and analyzed using Quantity-One software (version 4.6.2, Bio-Rad, Hercules, USA). The similarities of the tested samples, as well as the simulative mean chromatogram were calculated and generated using the professional software named "Similarity Evaluation System for Chromatographic Fingerprint of Traditional Chinese Medicine" (Matlab version, Ver1.315, developed by the Research Center of Modernization of Chinese Herbal Medicine, Central South University and the Hong Kong Polytechnic University).

3. Results and discussion

3.1. Optimization of gel electrophoresis

PACE is a good method for analysis of oligosaccharides with reducing ends derived from polysaccharides, and gel electrophoresis conditions such as fluorophores used for derivatization of sugars, percentage of acrylamide in gels, and electrophoresis buffers were optimized (Goubet et al., 2002). Actually, the migration of oligosaccharides was also dependent on the voltage. As shown in Fig. 2, the time of electrophoresis was decreased and the separation efficiency of oligosaccharides was enhanced with the increasing voltage. Eighteen bands were detected at voltage of 700 V, while 13 bands were found at 300 V. Indeed, electrophoresis of high molecular weight oligosaccharides need high voltage. However, high voltage (800 V) induced decreased resolution because of Joule heating. Therefore, voltage of 700 V was selected for analysis of partial acid and enzymatic hydrolysates of polysaccharides from *Ganoderma*. Generally, the main final products of dextranase (EC 3.2.1.11) hydrolysates of dextran were isomaltose (DP2) and isomaltotriose (DP3) (Wu, Zhang, Huang, & Hu, 2011). In order to determine the DP of oligosaccharides in hydrolysates, both partial acid and dextranase hydrolysates ladders of dextran were used. In addition, bromophenol blue (black band) was selected as the migration indicator. The results indicated that the migration of bromophenol blue

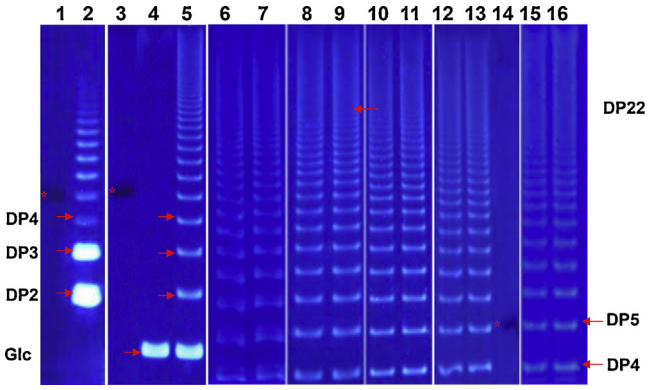

Fig. 2. PACE analysis of the hydrolysates of dextran with different voltage. Lanes 1 and 3, bromophenol blue at 700 V; lane 2, dextranase (EC 3.2.1.11) hydrolysates of dextran at 700 V; lane 4, glucose at 700 V; lanes not mentioned, partial acid hydrolysates of dextran at 800 V (6 and 7), 700 V (5, 8 and 9), 600 V (10 and 11), 500 V (12, 13 and 14), 300 V (15 and 16), respectively.

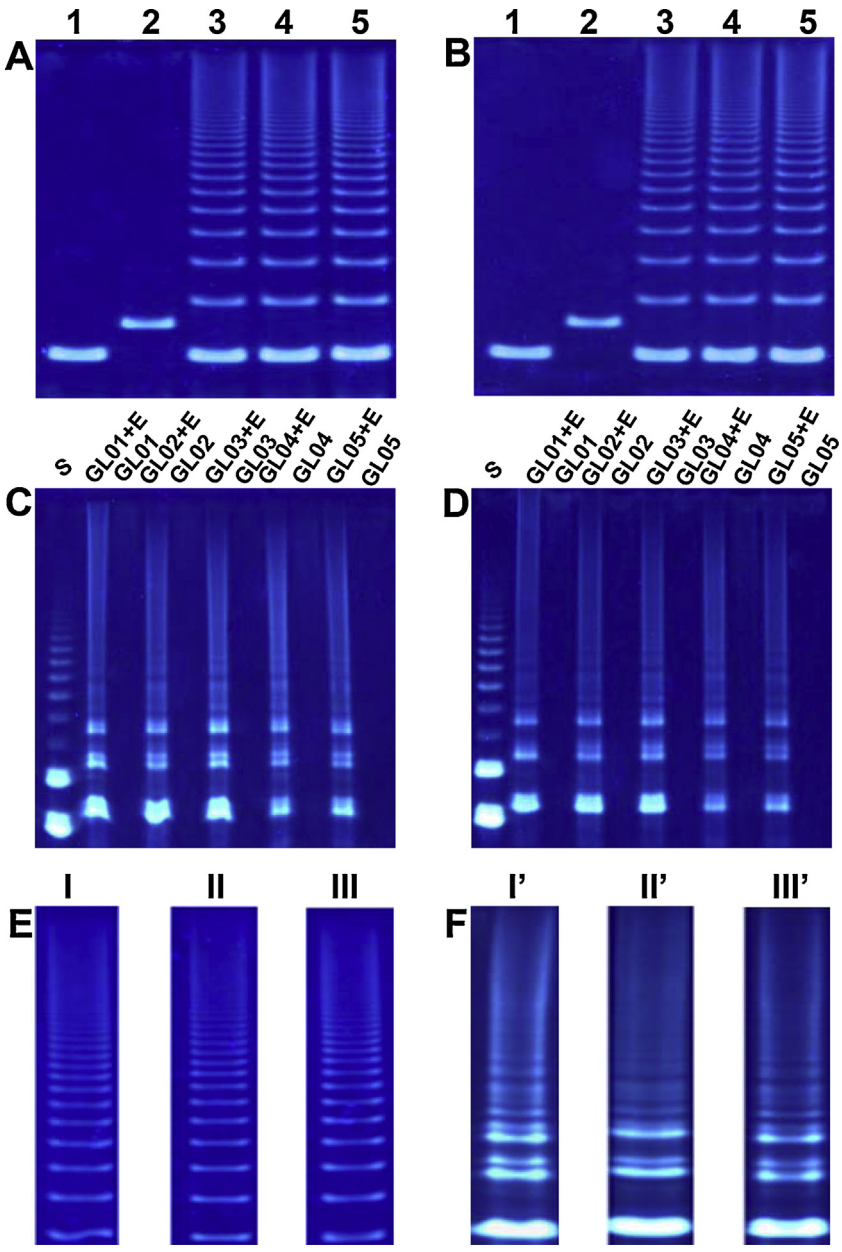

Fig. 3. PACE repeatability of partial acid hydrolysis of dextran (A and B), endo-1,3-β-D-glucanase digested polysaccharides from GL01-GL05 (C and D) as well as the stability of partial acid hydrolysates of dextran (E) and endo-1,3-β-D-glucanase hydrolysates of polysaccharides from sample GS09 (F). Lane 1, glucose; lane 2, lactose; lanes 3–5, partial acid hydrolysates of dextran; lane S, dextranase hydrolysate of dextran used as markers; lanes I and I′, II and II′, III and III′, sample stored for 1, 3 and 5 days at −20 °C, respectively. E, endo-1,3-β-D-glucanase; GL01–GL05 and GS09, same as in Table 1.

(marked as asterisk) was very similar to the DP5 oligosaccharide (Fig. 2).

Fig. 3 showed the method repeatability and sample stability, which indicated that the method repeatability was good and the samples stored at −20 °C were stable. Actually, the samples could be stable for at least 6 months at −20 °C (Goubet et al., 2011).

3.2. Partial acid hydrolysates profiles of polysaccharides from Ganoderma and adulterants

High-performance thin-layer chromatography (HPTLC) based on acid hydrolysates of polysaccharides were developed for discrimination and comparison of polysaccharides from *Ganoderma* (Di et al., 2003; Xie et al., 2012). However, HPTLC is usually not suitable for analysis of saccharides with high DP. Moreover, the sensitive of coloration for HPTLC are usually poor. PACE has the advantages of high resolution (oligosaccharides with a DP of 40 can be separated) and good sensitivity (as little as 100 fmol of oligosaccharides can be detected) (Goubet et al., 2002; Goubet, Morriswood, & Dupree, 2003). Therefore, in this study, the partial acid hydrolysates of polysaccharides from *Ganoderma* and some adulterants were investigated using PACE (Fig. 4). Similar to the results of HPTLC analysis (Di et al., 2003; Xie et al., 2012), PACE profiles of partial acid hydrolysates of polysaccharides from *G. lucidum* and *G. sinense* were also similar, but their fingerprints were obviously different from those of other species of *Ganoderma* (GT, GC, GA, and GSM) and adulterants (LR, FF, IO and AM). It is interesting that PACE profiles of some samples, such as GTC (lane 5) and TV (lane 9), LR (lane 3) and GT (lane 4), GA (lane 11) and IO (lane 12), as well as FF (lane 6), GC (lane 7), GSM (lane 8) and AM (lane 10) were similar, respectively, which could be discriminated from Lingzhi (Fig. 4). However, acid hydrolysis is non-specific. Therefore,

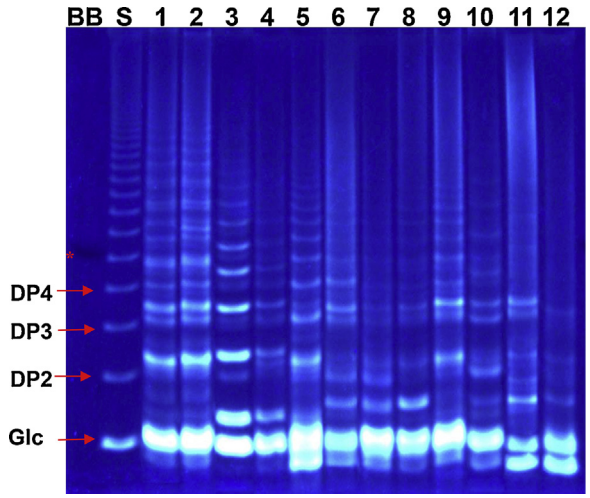

Fig. 4. PACE analysis of partial acid hydrolysates of polysaccharides from *Ganoderma* and adulterants. Lane BB, bromophenol blue; lane S, partial acid hydrolysates of dextran used as markers; lane 1–lane 12, sample of GL08, GS09, LR, GT, GTC, FF, GC, GSM, TV, A1, GA and IO, respectively. The sample codes were the same as in Table 1.

enzymatic hydrolysis based on chemical structural characters of polysaccharides should be performed.

3.3. Enzymatic digested profiles of polysaccharides from Ganoderma and adulterants

3.3.1. Endo-1,3-β-D-glucanase digested polysaccharides fingerprints

Enzymatic digestion is a specific and mild hydrolysis approach with high selectivity, which has been used for discrimination and characterization of polysaccharides from traditional Chinese medicines in our group (Guan & Li, 2010; Guan et al., 2011; Xie et al., 2012; Xu, Guan, et al., 2011). Previous studies have shown that polysaccharides from Lingzhi usually consist of arabinose, galactose, glucose and xylose, and the major bioactive polysaccharide is 1,3-linked β-D-Glc*p* with 1–15 units of 1,6-linked β-D-Glc*p* side chains (Paterson, 2006). Other linkages such as 1,4-linked β-D-Glc*p* and 1,6-linked Man*p* are also existed in polysaccharides of Lingzhi (Gao et al., 2009; Han, Chan, Yu, et al., 2012; Han, Chan, Dong, et al., 2012). Additionally, pectinase had obvious effects on polysaccharides from Lingzhi, but specific characteristics were not found due to a single column HPSEC was not suitable for simultaneous separation of both polysaccharides and their hydrolysates (Guan & Li, 2010; Xie et al., 2012). Therefore, endo-1,3-β-D-glucanase and pectinase were selected for enzymatic digestion of polysaccharides from *Ganoderma* spp. and adulterants based on our previous studies (Guan & Li, 2010; Xie et al., 2012).

The responses of polysaccharides to endo-carbohydrase are dependent on their sugar composition and linkage (Barton et al., 2006). PACE fingerprints of endo-1,3-β-D-glucanase digested polysaccharides from Lingzhi are shown in Fig. 5. The results showed that polysaccharides from Lingzhi, *G. lucidum* and *G. sinense*, could be completely digested to produce small sugars, with DP between 2 and 4, which were not exist in samples before enzymatic hydrolysis (Fig. 5). The results suggested the main structure of polysaccharides from Lingzhi were 1,3-β-linked glucan, which was in accordance with the previous report (Paterson, 2006). Actually, migration of saccharides depended on their compositional monosaccharides and glucosidic linkage except the molecular size and charge (Goubet et al., 2011, 2002). For example, 1,3-β-oligoglucan can be separated from a 1,4-β-oligoglucan with the same DP (Goubet et al., 2011), as well as lactose and isomaltose (Fig. 3). Moreover, chromatogram of each sample was obtained based on the gel image using Quantity-One software. The similarities of their entire chromatographic patterns were evaluated by using "Similarity Evaluation System for Chromatographic Fin-

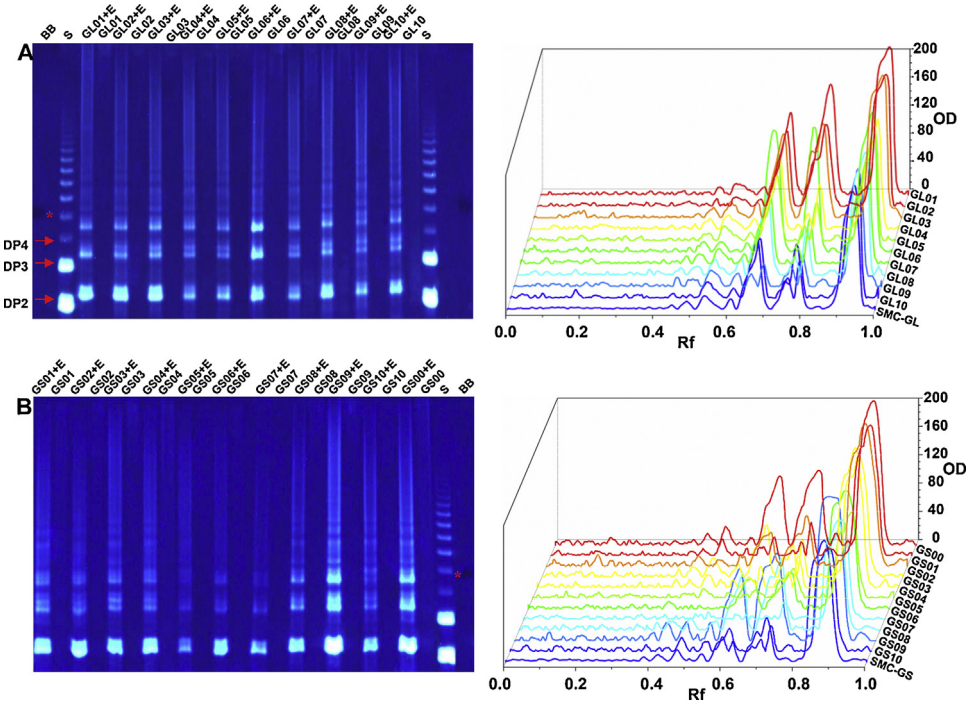

Fig. 5. PACE fingerprints (left) and chromatograms (right) of endo-1,3-β-D-glucanase (E) digested polysaccharides from *G. lucidum* (upper) and *G. sinense* (lower). The sample codes were the same as in Table 1. BB, bromophenol blue; S, dextranase digested dextran used as markers; SMC-GL and SMC-GS, the simulative mean chromatograms of *G. lucidum* and *G. sinense*, respectively.

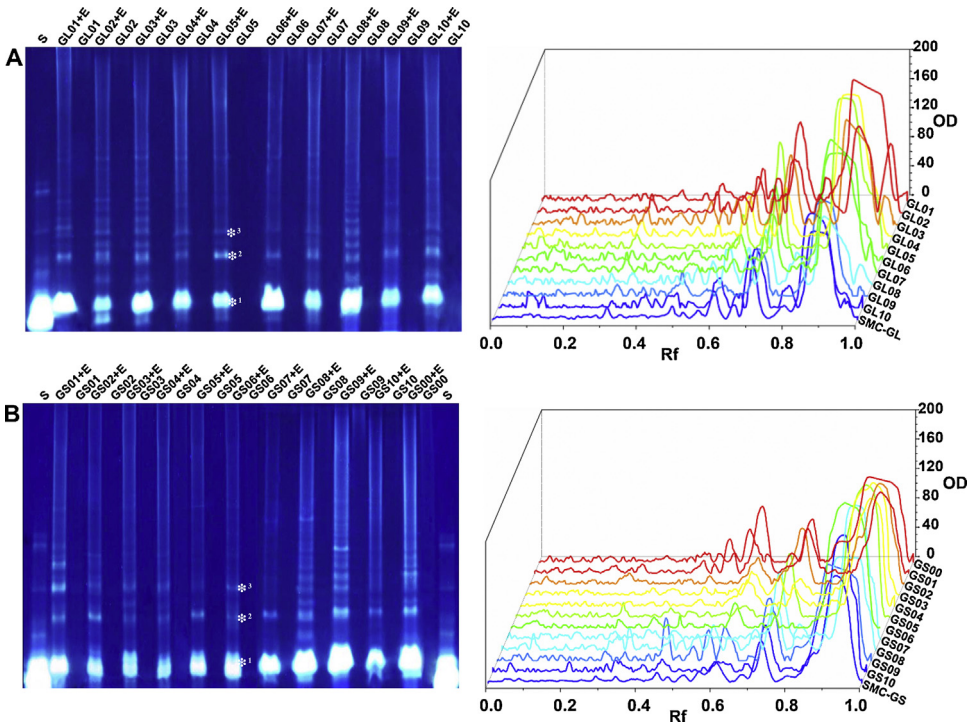

Fig. 6. PACE fingerprints (left) and chromatograms (right) of pectinase (E) digested polysaccharides from *G. lucidum* (upper) and *G. sinense* (lower). S, pectinase digested polygalacturonan used as markers; SMC-GL and SMC-GS, the simulative mean chromatograms of *G. lucidum* and *G. sinense*, respectively. The sample codes were the same as in Table 1.

gerprint of Traditional Chinese Medicine". The average correlation coefficient of each chromatogram to their simulative mean chromatogram (SMC-GL) was 0.960 ± 0.023 ($n = 10$), which supported the enzymatic fingerprints of polysaccharides from *G. lucidum* were pretty similar. Finally, a representative sample (GL08) was selected as reference for comparison with other species of *Ganoderma* and adulterants. The enzymatic fingerprints of different samples of *G. sinense* were also very similar, and average correlation coefficient of each chromatogram to their simulative mean chromatogram (SMC-GS) was 0.969 ± 0.012 ($n = 11$). Therefore, sample of GS09 was used as reference for discrimination with the other species of *Ganoderma* and adulterants. It is interesting that the polysaccharides from *G. lucidum* and *G. sinense* were similar (Fig. 5), and the correlation coefficient between *G. sinense* (GS09) and *G. lucidum* (GL08) was 0.961.

3.3.2. *Pectinase digested polysaccharides fingerprints*

PACE fingerprints of pectinase digested polysaccharides from Lingzhi were shown in Fig. 6. The results showed that all tested samples of Lingzhi could be hydrolyzed by pectinase, which supported our previous report (Xie et al., 2012) and suggested that polysaccharides from Lingzhi richly contained 1,4-α-D-galactosiduronic linkages. However, the difference of enzymatic profiles of polysaccharides in Lingzhi from different regions was obvious (Fig. 6). Actually, pectins were a group of polysaccharides, including polygalacturonan, rhamnogalacturonan I, rhamnogalacturonan II and xylogalacturonan (Brink & Vries, 2011). The difference might attribute to the content variation of different types of pectins in Lingzhi from different regions. The similar phenomenon was also found in pectinase digested polysaccharides from *Cordyceps* (Guan et al., 2011). These differences might be available for discrimination of herbal polysaccharides from different locations. The average correlation coefficient of individual chromatogram, obtained based on the gel images, of *G. lucidum* and *G. sinense* to their SMC-GL and SMC-GS were 0.943 ± 0.049 ($n = 10$) and 0.936 ± 0.047 ($n = 11$), respectively.

3.4. *Comparison of polysaccharides from Ganoderma spp. and adulterants*

HPTLC analysis showed that acidic hydrolysates of polysaccharides from *G. lucidum* and *G. sinense* (Xie et al., 2012), as well as *G. lucidum*, *G. nigrolucidum*, *G. applanatum* and *G. tropicum* (Di et al., 2003) were similar. But acid hydrolysis was not specific. Enzymatic digestions followed by PACE analysis showed the difference among various species of *Ganoderma*, especially the adulterants, were obvious, though few similar bands were also found in different samples (Fig. 7). Briefly, polysaccharides from LR and GT could not be hydrolyzed by both endo-1,3-β-D-glucanase and pectinase, while FF and IO could not be hydrolyzed by pectinase. Therefore, they could be directly discriminated from Lingzhi (GL08 and GS09) based on enzymatic fingerprints of endo-1,3-β-D-glucanase and pectinase. In addition, polysaccharides from GSM, GC, GTC and TV could be hydrolyzed by both endo-1,3-β-D-glucanase and pectinase, but their fingerprints were obviously different from those of Lingzhi. It is interesting that profile of endo-1,3-β-D-glucanase digested polysaccharides from FF and AM were similar with those of Lingzhi, but their pectinase hydrolysates were obviously different (Fig. 7). Especially, polysaccharides from cultured rather than natural GT could be digested by endo-1,3-β-glucanase and pectinase, which might attribute to the different environmental conditions could induce various polysaccharide formation (Yang & Liau, 1998).

The correlation coefficient of individual chromatogram of *Ganoderma* and commercial adulterants to Lingzhi (GL 08 and GS 09) were summarized in Table 2. The results showed that GSM and GTC, then AM and TV, had higher similarity to Lingzhi. But natural GT and GA were obviously different

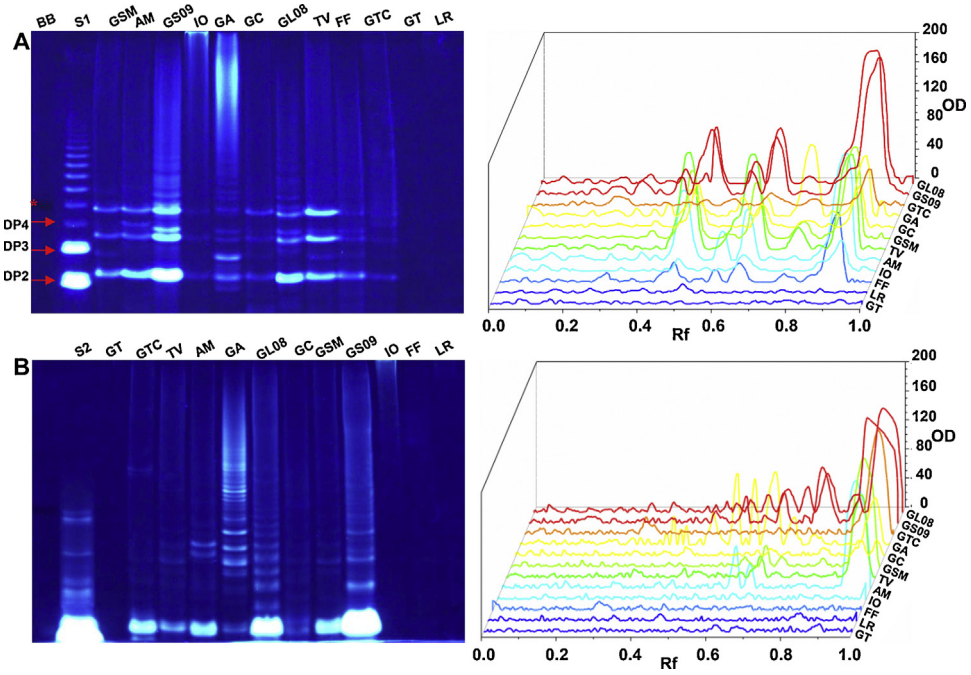

Fig. 7. PACE fingerprints (left) and chromatograms (right) of endo-1,3-β-D-glucanase (upper) and pectinase (lower) digested polysaccharides from *Ganoderma* and adulterants. BB, bromophenol blue; S1, dextranase digested dextran used as markers. S2, pectinase digested polygalacturonan used as markers. The sample codes were the same as in Table 1.

Table 2
The correlation coefficient of each tested sample to the representative Lingzhi.

	Samples											
	GL08	GS09	GSM	GA	GC	GT	GTC	AM	TV	FF	LR	IO
Endo-1,3-β-D-glucanase hydrolysates												
CC-GL08-G[a]	1.000	0.961	0.873	0.459	0.715	0.158	0.807	0.923	0.754	0.938	0.082	0.907
CC-GS09-G[b]	0.961	1.000	0.886	0.422	0.716	0.140	0.742	0.923	0.759	0.913	0.032	0.887
Pectinase hydrolysates												
CC-GL08-P[a]	1.000	0.901	0.936	0.388	0.580	0.100	0.900	0.899	0.859	0.040	0.039	0.047
CC-GS09-P[b]	0.901	1.000	0.767	0.273	0.502	0.245	0.777	0.709	0.678	0.012	0.090	0.012

GL08, *Ganoderma lucidum*; GS09, *Ganoderma sinense*; GSM, *Ganoderma sessile* Murr.; GA, *Ganoderma applanatum*; GC, *Ganoderma capense*; GT, *Ganoderma tropicum*; GTC, *Ganoderma tropicum* (cultured); AM, *Amauroderma* spp.; TV, *Trametes versicolor*; FF, *Fomes fomentarius*; LR, *Lignosus rhinoceros*; IO, *Inonotus obliquus*.

[a] Similarity compared with *G. lucidum*.

[b] Similarity compared with *G. sinense*.

from Lingzhi, which was not inaccordance with the results derived from acidic hydrolysates fingerprinting (Di et al., 2003).

4. Conclusions

The saccharide mapping, enzymatic digestion followed by PACE analysis, is a good method with high repeatability and stability for analysis of polysaccharides from *Ganoderma* spp. and adulterants, which is helpful to improve the quality control of polysaccharides from *Ganoderma*. However, in order to clearly differentiate Lingzhi and its adulterants, much larger sample size is necessary for further study.

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