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Characterization of an antiproliferative exopolysaccharide (LHEPS-2) from *Lactobacillus helveticus* MB2-1

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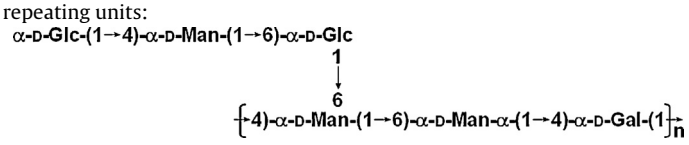
ABSTRACT

In this study, we reported that three purified exopolysaccharides fractions (LHEPS-1, LHEPS-2 and LHEPS-3) and crude LHEPS from the *Lactobacillus helveticus* MB2-1 inhibited the proliferation potential of human gastric cancer BGC-823 cells. We found that all the LHEPS fractions and crude LHEPS exerted concentration and time dependent inhibitory activities *in vitro* on BGC-823 cells. LHEPS-2 exhibited higher antiproliferative activity against BGC-823 cells *in vitro* than LHEPS-1, LHEPS-3 and crude LHEPS. With the consideration of the outstanding antiproliferative effect of LHEPS-2 on BGC-823 cells, more fine structural characterization of LHEPS-2 was further identified. The structure of LHEPS-2 was elucidated by methylated analysis, GC–MS, ^{1}H NMR and ^{13}C NMR spectroscopy, together with two-dimensional (2D) COSY, TOCSY, HSQC, HMBC and NOESY spectroscopy. Results showed that the LHEPS-2 was consisted of the following repeating units:



1. Introduction

In spite of recent advances in diagnostic and therapeutic techniques, gastric cancer is still the second and third leading causes of cancer-related death worldwide, for females and males, respectively (Chen et al., 2013; Jemal et al., 2011). 5-Fluorouracil (5-FU) is widely used to treat gastric cancer, but its side effects, such as nausea, vomiting and fatigue, disturbed the quality of patients' lives (Adamsen et al., 2006; Wagner et al., 2006). Therefore, searching for new alternative strategies for the prevention and treatment of gastric cancer is essential. Recently, a wide variety of natural products have been recognized to have the ability to induce apoptosis in various tumor cells of human origin and many of these substances are from plants, animals and microorganisms sources with low or no toxicities to human body (Gan, Ma, Jiang, Xu, & Zeng, 2011; Goodarzi, Varshochian, Kamalinia, Atyabi, & Dinarvand, 2013; Qiao et al., 2010).

For thousands of years, lactic acid bacteria (LAB) have been known as an important source of nutritional diet and medicine. Extensive studies have revealed that many species of LAB have promising potential in improving human health and preventing diseases (Axelsson, 2004; Li, Mutuvulla, Chen, Jiang, & Dong, 2012). Exopolysaccharides (EPS), one of the most important components isolated from LAB, have been correlated with multiple pharmacological activities, such as antioxidant, anticancer, immunomodulatory and cholesterol lowering activities (Liu et al., 2011; Pan & Mei, 2010; Suresh et al., 2013; Yang et al., 2012). Though the mechanism of polysaccharide's anticancer activity is unknown yet, many research works have been done. Recently, several polysaccharides have been reported capable of inducing apoptosis *via* reactive oxygen species (ROS) production in mitochondria (Goodarzi et al., 2013; Zong, Cao, & Wang, 2012). Oxidative stress plays a crucial role in the pathogenesis of cancer. Growing evidences indicate that the level of antioxidation and reactive oxygen species are well correlated with the generation and malign transformation of cancer cells (Jiang, Wang, Liu, Gan, & Zeng, 2011; OuYang et al., 2013). If polysaccharides can enhance the level of antioxidation and clear the reactive oxygen species in cancer cells, they may inhibit the growth of cells (He, Yang, Jiao, Tian, & Zhao, 2012).

Recently, we reported that the *Lactobacillus helveticus* MB2-1 exopolysaccharides (LHEPS) were fermented with whey medium

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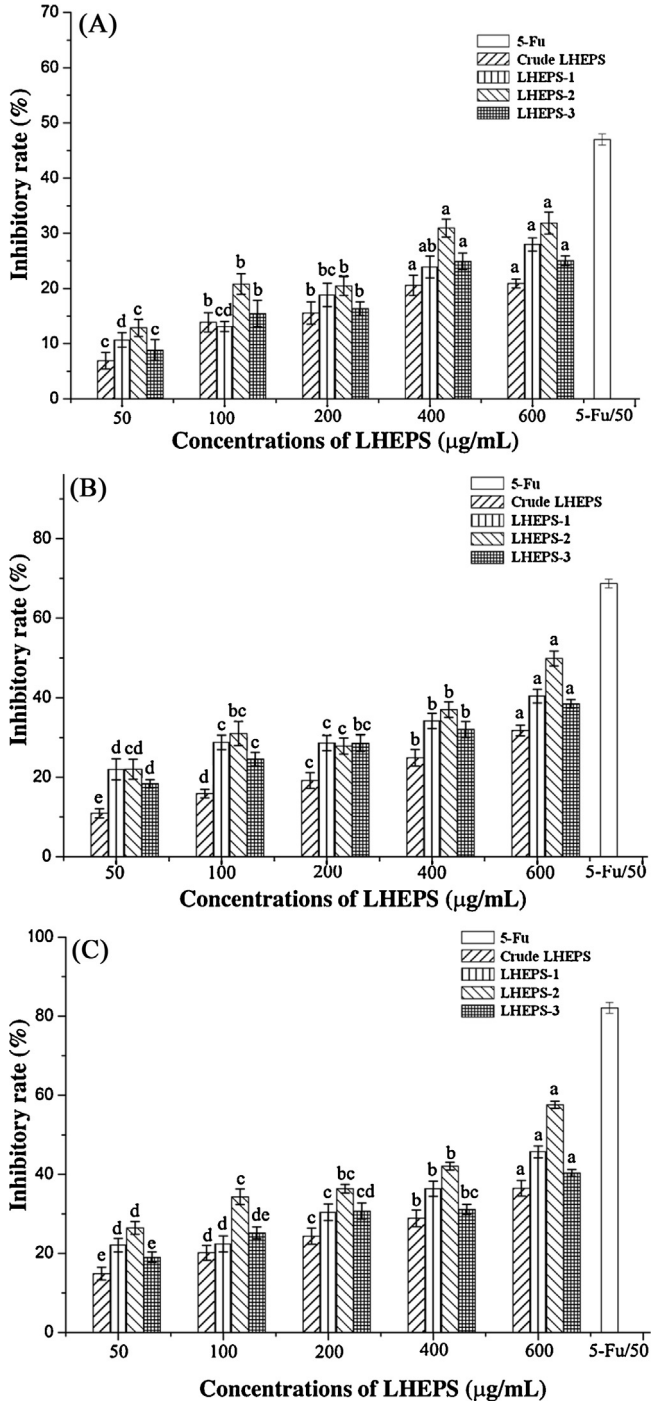

Fig. 1. Inhibitory effects *in vitro* of LHEPS-1, LHEPS-2, LHEPS-3, crude LHEPS and 5-Fu against human gastric cancer BGC-823 cells at 24 h (A), 48 h (B) and 72 h (C) treatment. Data are means ± SD of triplicate. [a]Different lowercases indicate significant differences ($P < 0.05$) for the same LHEPS fractions and crude LHEPS at different concentrations, respectively.

and further fractionated on DEAE-52 cellulose and Sephadex G-100 chromatography to afford three purified fractions of LHEPS-1, LHEPS-2 and LHEPS-3. Then, their preliminary structures and antioxidant activities *in vitro* were investigated. Three purified EPS fractions (LHEPS-1, LHEPS-2 and LHEPS-3) had similar molecular weight distributions (about 2×10^5 Da), and they were composed of galactose, glucose and mannose with different molar ratio. In addition, the antioxidant activity of LHEPS-1, LHEPS-2 and LHEPS-3 was evaluated with the *in vitro* scavenging abilities. The results demonstrated that LHEPS-2 and LHEPS-3 showed stronger scavenging activities on three kinds of free radicals and more potent metal ion chelating activity than that of LHEPS-1 (Li et al., 2012; Li, Ji, et al., 2013; Li, Dobruchowska, Gerwig, Dijkhuizen, & Kamerling, et al., 2013). Till now, there have been many reports on the antioxidant and anticancer activities of polysaccharides derived from plants and animals (Goodarzi et al., 2013; Liu et al., 2009; Qiao et al., 2010; Xu, Ye, Sun, Tu, & Zeng, 2012). However, few investigations are focused on the antioxidant and anticancer effects of EPS from LAB on the proliferation of human gastric cancer cells (BGC-823).

In our research, crude LHEPS and three purified LHEPS fractions from *L. helveticus* MB2-1 was obtained. Then the antiproliferative effects with BGC-823 cells of crude LHEPS and different LHEPS fractions *in vitro* were determined. Meanwhile, to pursue the structure–activity relationship of LHEPS fraction with high antiproliferative activity, the structure of LHEPS-2 was elucidated by using methylated analysis, gas chromatography–mass spectroscopy (GC–MS), nuclear magnetic resonance spectroscopy (NMR), including ^{1}H NMR and ^{13}C NMR, together with two-dimensional (2D) ^{1}H–^{1}H correlated spectroscopy (COSY), ^{1}H–^{1}H total correlation spectroscopy (TOCSY), ^{1}H–^{13}C heteronuclear single quantum coherence (HSQC), ^{1}H–^{13}C heteronuclear multiple quantum coherence (HMBC) and nuclear Overhauser effect (NOESY) spectroscopy.

2. Materials and methods

2.1. Materials

The strain *L. helveticus* MB2-1 was isolated from traditional Sayram ropy fermented milk that was collected from Sayram town of Baicheng in Xinjiang vygur autonomous region of China (Li et al., 2012). Human gastric cancer BGC-823 cells were obtained from the Cell Bank of Shanghai Institute of Cell Biology (Shanghai, China). 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT), fetal bovine serum (FBS) and Dulbecco's modified Eagle's medium (DMEM) were purchased from Gibco (Gibco BRL, Grand Island, NY, USA). Dimethyl sulfoxide (DMSO), 5-Fu, penicillin and streptomycin were obtained from Sigma Chemical Co. (St. Louis, MO, USA). All other reagents were of analytical grade.

2.2. Isolation and purification of LHEPS

The LHEPS was isolated and purified according to our previously method (Li et al., 2012; Li, Ji, et al., 2013; Li, Dobruchowska, et al., 2013). Briefly, after 24 h of incubation period, the cell was separated by centrifugation at 12,000 × *g* for 15 min at 4 °C. Then trichloroacetic acid (TCA) solution was added to the culture to give a final concentration of 4% (w/v), and the precipitated proteins were removed by centrifugation (12,000 × *g* for 30 min at 4 °C). The supernatant was filtered through a 0.45 μm membrane filter, mixed with three volumes ice cold ethanol, stirred vigorously and kept at 4 °C for overnight. Crude LHEPS was collected by centrifugation at 15,000 × *g* for 15 min. The LHEPS pellet was dissolved in distilled water and dialyzed (Mw cut-off 8000–14,000 Da, Solarbio Co. Ltd, Beijing, China) against distilled water for 48 h at 4 °C and then lyophilized.

The freeze-dried sample was fractionated with an anion exchange chromatography on the DEAE-cellulose column (2.6 cm × 30 cm), eluted with a step gradient of NaCl solution (0, 0.1 and 0.3 M) at a flow rate of 60 mL/h. The fractions were assayed for carbohydrate content by the phenol–sulfuric acid method. Peak fractions containing LHEPS were pooled, dialyzed

and lyophilized. Further purification of the LHEPS was performed by gel filtration using a Sephadex G-100 column (2.6 cm × 100 cm) eluted with distilled water at a flow rate of 12 mL/h. The three purified LHEPS fractions (LHEPS-1, LHEPS-2 and LHEPS-3) were pooled, dialyzed with water and freeze-dried for antiproliferative activity study.

2.3. Antiproliferative activity of LHEPS

The inhibitory rates of crude LHEPS and its purified fractions on the growth of human gastric cancer BGC-823 cells were determined by MTT-based colorimetric method previously reported (Wang et al., 2013). Briefly, BGC-823 cells were suspended in DMEM medium supplemented with 10% FBS, 100 U/mL of penicillin and 100 mg/L of streptomycin at a density of 2×10^5 cells/mL. The cell suspension was pipetted into a 96-well plate (100 μL/well) and inoculated at 37 °C in a humidified 5% CO_2 incubator (Thermo Electron, Waltham, MA) for 24 h. Then, 50 μL of test sample with different concentrations (0, 50, 100, 200, 400 and 600 μg/mL in fresh growth medium) and 5-FU (50 μg/mL) were added into each well for 24, 48 and 72 h, respectively. At the end of each treatment, MTT reagent (5.0 mg/mL) was added to each well (10 μL/well), and the plate was further incubated for 4 h. The liquid was then removed and 100 μL DMSO was added to the well. After dissolving of the formed crystal formazan, the absorbance was measured by a Synergy™-2 microplate reader (BioTek Instruments, Inc., Burlington, VT) at 570 nm. The inhibitory rate was calculated according to the following formula:

$$\text{Inhibitory rate}\,(\%) = \left[\frac{1-(A_{\text{sample}}-A_{\text{blank}})}{A_{\text{control}}-A_{\text{blank}}}\right] \times 100$$

where A_{control} and A_{blank} were the absorbance of the system without the addition of samples and cells, respectively.

2.4. Methylation and GC–MS analysis for LHEPS-2

Methylation and GC–MS analysis of LHEPS-2 were performed by using the reported procedure (Liu et al., 2009). Complete methylation was confirmed by the disappearance of O–H absorption band (3700–3100 cm^{-1}) in Fourier-transform infrared (FT-IR) spectrum. The permethylated LHEPS-2 was hydrolyzed with 2 M trifluoroacetic acid (TFA) at 120 °C for 2 h (Wack & Blaschek, 2006). Then the methylated sugars were reduced with $NaBH_4$ for 2 h and acetylated with pyridine-acetic anhydride (1:1, v/v) at 100 °C for 1 h. The resulting partially methylated alditol acetate derivatives were analyzed by GC–MS. GC–MS analysis was performed on a Agilent 5975MSD-6890 GC–MS (Agilent Technologies, CA, USA), equipped with a HP-5 capillary column was used with He as carrier gas. The oven temperature was initially set at 100 °C, programmed at 6 °C/min to 250 °C and held at 250 °C for 5 min.

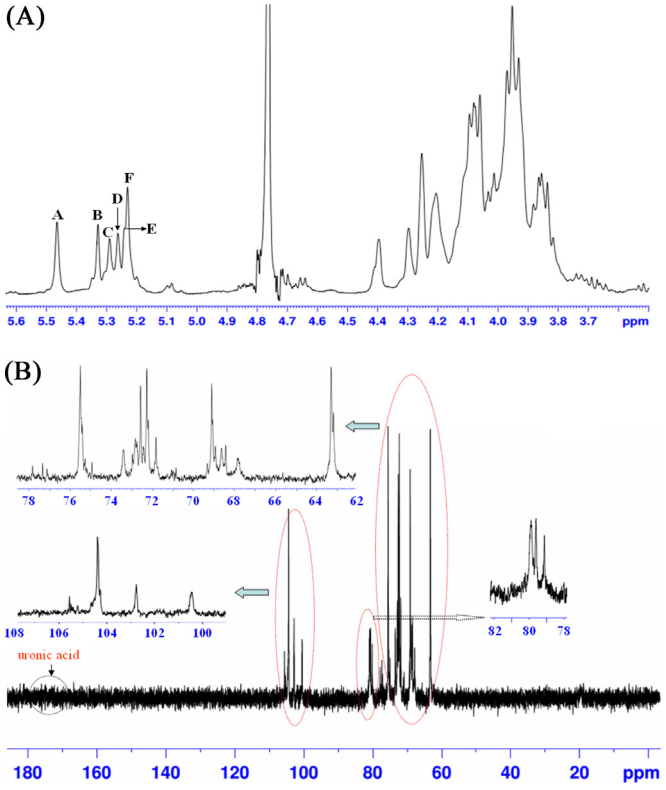

Fig. 2. The 500-MHz ^1H and ^{13}C NMR spectra of LHEPS-2 from *L. helveticus* MB2-1 recorded on Bruker AVANCE AV-500 spectrometer in D_2O. ^1H NMR spectrum of LHEPS-2 (A); ^{13}C NMR spectrum of LHEPS-2 (B).

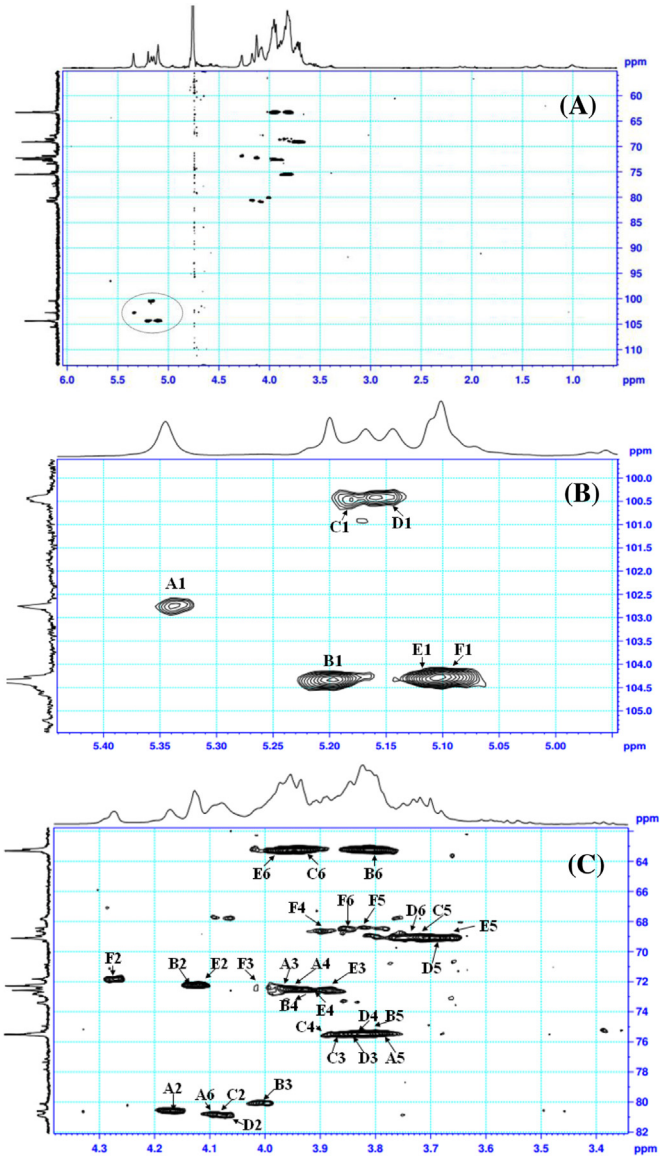

Fig. 3. ^1H–^{13}C HSQC spectrum of LHEPS-2 from *L. helveticus* MB2-1 recorded on Bruker AVANCE AV-500 spectrometer in D_2O (A). The assignments of sugar residues in anomeric region (B); the assignments of sugar residues in non-anomeric region (C).

2.5. NMR analysis

The structure of LHEPS-2 (50 mg/mL) was identified by NMR. ^{1}H NMR, ^{13}C NMR and 2D spectra were recorded in D_2O (99.9 at.% D, Cambridge Isotope Laboratories, Inc., Andover, MA, USA) as solvent at 323 K with a Bruker AVANCE AV-500 spectrometer (Bruker Group, Fällanden, Switzerland) using the residual solvent signal as internal standard. The chemical shifts (δ) are given in parts per million (ppm), and coupling constants (J) are given in Hz. The 2D COSY, TOCSY, HSQC, HMBC and NOESY measurements were used to assign signals and to determine the sequence of sugar residues.

2.6. Statistical analysis

Statistical analysis was performed using SPSS for windows, version 16.0. One-way analysis of variance (ANOVA) and the Fisher's least significant difference (LSD) test were used to determine significant differences for different samples. Differences were considered to be significant when $P < 0.05$. Data were obtained from three independent experiments and each sample was analyzed in triplicate.

3. Results and discussion

3.1. Antiproliferative activity in vitro of LHEPS

Human gastric cancer line BGC-823 is an ideal model for the study of cell proliferation (Gan et al., 2011). There have been few reports regarding antiproliferative activities of the EPS obtained from LAB. In the present study, therefore, the antiproliferative activity of LHEPS *in vitro* was investigated. As shown in Fig. 1, all the purified LHEPS fractions and crude LHEPS exerted inhibitory activities *in vitro* on BGC-823 cells with concentration and time dependent manners. After 24 h treatment, LHEPS-2 exhibited the strongest antiproliferative activity (inhibitory rate, 31.84%) at a dose of 600 μg/mL, followed by LHEPS-1, LHEPS-3 and crude LHEPS with the corresponding inhibition rates of 27.97%, 25.04% and 20.91%, respectively (Fig. 1A). Furthermore, the effects of exposure time by LHEPS-1, LHEPS-2, LHEPS-3 and crude LHEPS toward BGC-823 cells were investigated. Compared with those of 24 and 48 h exposure times, there were significant increases ($P < 0.05$) of inhibitory rate for the samples with 72 h exposure time. After 72 h incubation, the inhibition rates at a concentration of 600 μg/mL for LHEPS-2, LHEPS-1 and LHEPS-3 were 57.58%, 45.69% and 40.33%, respectively (Fig. 1C). However, the antiproliferative activity of crude LHEPS was no more than 36.50% regardless the changes of dose and exposure time. Apparently, LHEPS-2 possessed a relative higher antiproliferative activity *in vitro* than LHEPS-1, LHEPS-3 and crude LHEPS. In our previous report, we also demonstrated the purified LHEPS-2 with relative stronger scavenging activity *in vitro* on three kinds of free radicals and chelating activity on ferrous ion. These results suggested that LHEPS-2 might be a potential natural drug or health food for gastric cancer therapeutics. Considering the outstanding antiproliferative and antioxidant effects on BGC-823 with LHEPS-2, more fine structure characterization of LHEPS-2 was further identified based on our previous data (Li, Ji, et al., 2013; Li, Dobruchowska, et al., 2013).

3.2. Methylation analysis of LHEPS-2

For determination of the linkage of monosaccharides, LHEPS-2 was methylated, hydrolyzed and converted into their corresponding alditol acetates for GC–MS analysis. The individual peaks of the methylated sugar residue and fragmentation patterns were identified by their retention time in GC and by comparison with literature mass spectra patterns (Charchoghlyan & Park, 2012; Górska et al., 2010; Notararigo et al., 2013; Rodríguez-Carvajal et al., 2008; Yin et al., 2012; Zhang et al., 2012). As summarized in Table 1, in LHEPS-2, six glycosyl residues were connected to form one polysaccharide repeat unit. These were identified as alditol acetates derived from 2,3,6-tri-, 2,3,4-tri- and 2,3-di-O-methyl-D-mannose; 2,3,4-tri- and 2,3,4,6-tetra-O-methyl-D-glucose; 2,3,6-tri-O-methyl-D-galactose.

The molar ratio of the non-reducing T-D-Glc*p*-(1→ residue was 1.00. The majority of branched sugar residue was →4,6-D-Man*p*-(1→ with molar ratio of 1.01. These data indicated that the ratio between terminal unit and branching point was 0.99, which was consistent with the fact that the number of polysaccharide branching point approximately equals to the number of terminal unit. The unsubstituted residues were shown to be →4-D-Man*p*-(1→, →6-D-Man*p*-(1→, →4-D-Gal*p*-(1→ and →6-D-Glc*p*-(1→ in molar ratio of 1.30, 1.05, 1.21 and 1.13, respectively. The data from the methylation analysis suggested that LHEPS-2 was mainly composed of →4-D-Man*p*-(1→ and →6-D-Man*p*-(1→ as the backbone with some →4-D-Gal*p*-(1→ and →6-D-Glc*p*-(1→ which could exist in the backbone or side chains. The major branching points were at O-4 or O-6 positions of the Man*p* chain, with D-Glc*p* as the terminal residue. The subsequent NMR spectroscopy confirmed the conclusions drawn from methylation analysis and provided us with more details of the structure of LHEPS-2.

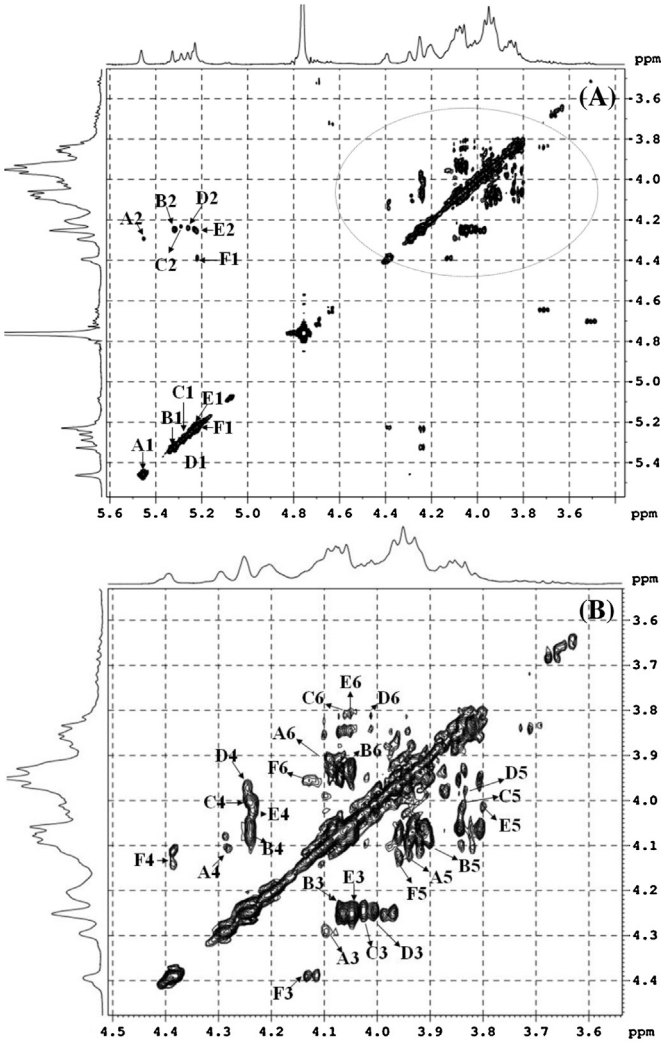

Fig. 4. ^{1}H–^{1}H COSY spectrum of LHEPS-2 from *L. helveticus* MB2-1 recorded on Bruker AVANCE AV-500 spectrometer in D_2O (A). The assignments of sugar residues in non-anomeric region (B).

Table 1
Methylation analysis data of LHEPS-2 from *L. helveticus* MB2-1.

Retention time (min)	Methylated sugar	Primary mass fragments (*m*/*z*)	Molar ratio	Deduced linkage
15.465	2,3,6-Me_3Man*p*	43.1, 68, 87, 117, 141.9, 161.1, 187.1, 213.1, 233.1, 277.3	1.30	→4-D-Man*p*-(1→
15.812	2,3,4-Me_3Man*p*	43.1, 65, 85, 117, 139, 159, 201.1, 231, 261.1, 281.9	1.05	→6-D-Man*p*-(1→
17.228	2,3,4,6-Me_4-Glc*p*	43.1, 71, 101.1, 129, 161.1, 205.1, 249, 355.2	1.00	T-D-Glc*p*-(1→
18.048	2,3,6-Me_3Gal*p*	43.1, 71, 101.1, 125, 145.1, 173.1, 205.1, 245.2, 281.1	1.21	→4-D-Gal*p*-(1→
18.996	2,3,4-Me_3Glc*p*	43.1, 87, 129, 159.1, 189.1, 233.1, 261, 355.1	1.13	→6-D-Glc*p*-(1→
19.078	2,3-Me_2Man*p*	43.1, 68, 87.1, 117, 142.1, 161.1, 189.1, 208.9, 233.1, 281	1.01	→4,6-D-Man*p*-(1→

3.3. ^{1}H NMR and ^{13}C NMR analysis of LHEPS-2

^{1}H NMR and ^{13}C NMR spectra of LHEPS-2 are shown in Fig. 2. Six obvious chemical shift signals of anomeric protons were found at δ 5.46, 5.33, 5.29, 5.26, 5.24 and 5.23 ppm in ^{1}H NMR spectrum. These results indicated that the LHEPS-2 contains six residues (named **A**, **B**, **C**, **D**, **E** and **F**). Based on their chemical shift and the value of the coupling constant for anomeric signals in the ^{1}H NMR spectrum, residues **A**–**F** were determined with α-configurations (the values of $J_{1,2}$ were 3–4 Hz). Combined with the result of monosaccharide analysis, the **A**–**F** residues may reflect six different α-type glycosidic bonds of galctose, glucose and mannose residues. Molar proportion of the six residues (**A**–**F**) was about 1.00:0.89:0.96:0.89:0.75:1.13 when estimated by the ratio of peak area of the integration of the H-1 signal for **A**–**F** residues. The corresponding H chemical shifts of residues **A**–**F** for other positions were packed in the range of δ 3.5–4.5 ppm and even difficult to discriminate. Similar results could be referred to some previous studies (Górska et al., 2010; Lebeer et al., 2009; Notararigo et al., 2013; Rodríguez-Carvajal et al., 2008). The ^{13}C NMR (Fig. 2B) chemical shifts in the area of anomeric carbon atoms also suggested six kinds of α-linkages for the galctose, glucose and mannose residues. The C-1 signals of the residues were detectable at δ 104.37, 104.29, 103.99, 102.75, 100.65 and 100.45 ppm. The existence of carbon atom signal in the range of 170–180 ppm proved that LHEPS-2 contains minor uronic acid or protein.

3.4. 2D-NMR analysis of LHEPS-2

The complete assignments of the ^{1}H and ^{13}C chemical shifts of the acid-hydrolyzed LHEPS-2 were carried out by means of the 2D ^{1}H–^{13}C HSQC, ^{1}H–^{1}H COSY, ^{1}H–^{1}H TOCSY, ^{1}H–^{13}C HMBC and NOESY experiments based on component analysis, FTIR analysis (data not shown) and on literatures data (Charchoghlyan & Park, 2012; Górska et al., 2010; Lebeer et al., 2009; Li, Ji, et al., 2013; Li, Dobruchowska, et al., 2013; Nie et al., 2011; Notararigo et al., 2013; Yin et al., 2010, 2012; Zhou et al., 2013). The single-bond correlations between the protons and the corresponding carbons obtained from HSQC spectra (Fig. 3) of LHEPS-2 in D_2O enabled all the ^{13}C NMR to be assigned. The C-1 signals at δ 104.37, 104.29, 103.99, 102.75, 100.65 and 100.45 ppm could be assigned to the **E**, **F**, **B**, **A**, **C** and **D**, respectively. These signals cross link to the proton signals at chemical shifts δ 5.24, 5.23, 5.33, 5.46, 5.29 and 5.26, respectively. The results from COSY (Fig. 4), based on stepwise magnetization transfers from the anomeric protons, could help assign the chemical shifts of other protons. The TOCSY spectrum provided all intraresidue connectivity of all protons of the **A**–**F**, respectively, as shown in Fig. 5. From the HMBC spectrum, the linkage of residue was obtained. Inspection of the NOESY spectrum showed on the **F** H-1 track a NOE connectivity with **D** H-6 in agreement with an **F**-(1→6)-**D** linkage. Inter-residue NOE cross-peaks between **D** H-1 and **A** H-6 supported the **D**-(1→6)-**A** linkage, indicating the branching position of residue **D** (data not shown). Thus when we combined the information from the ^{1}H and ^{13}C NMR, HSQC, COSY, TOCSY, HMBC and NOESY spectroscopy, a complete assignment of all the linkage patterns was identified as shown in Table 2. According to the results, it has been considered that residues **A**, **B**, **E** and **F** were substituted at C-6, C-4, C-4 and C-6 position, respectively. Thus, residues **A**, **B**, **E** and **F** belonged to →6-α-D-Man*p*-(1→, →4-α-D-Gal*p*-(1→, →4-α-D-Man*p*-(1→ and →6-α-D-Glc*p*-(1→ bond linked glycosides, respectively. In addition, residue **C** was assigned to a α-terminal-D-Glc and residue **D** was determined as the branching point belong to →4,6-D-Man*p*-(1→. An overall, structural unit of LHEPS-2 was proposed as shown in Fig. 6, and the value of '*n*' that repeat structural unit of LHEPS-2 was approximately equal to 214.

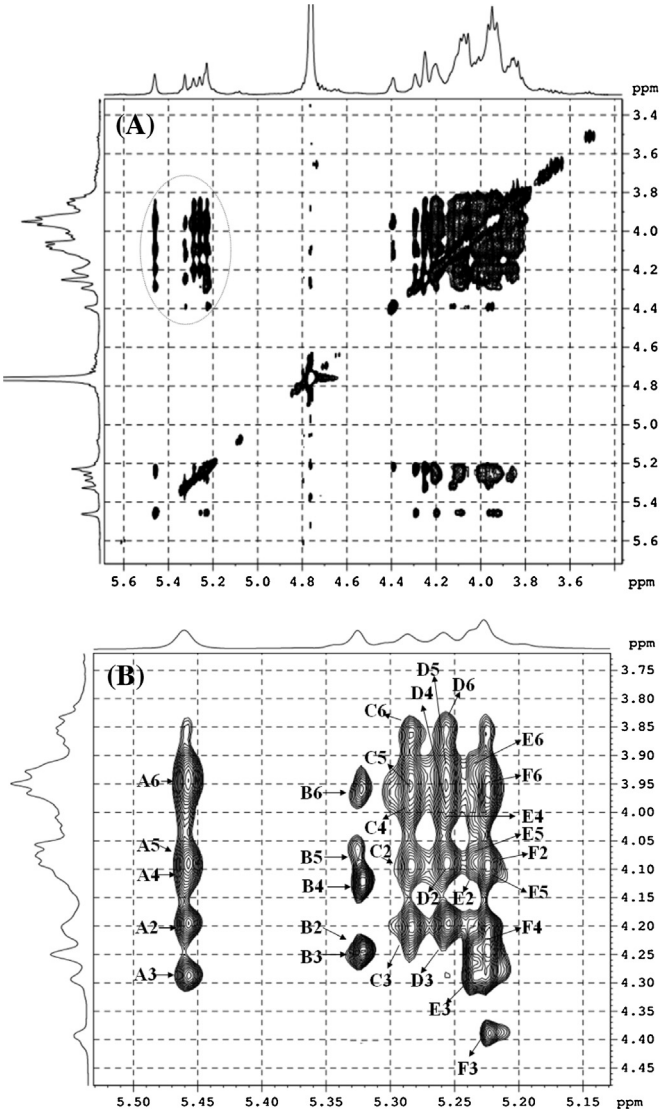

Fig. 5. ^{1}H–^{1}H TOCSY spectrum of LHEPS-2 from *L. helveticus* MB2-1 recorded on Bruker AVANCE AV-500 spectrometer in D_2O (A). The assignments of sugar residues in anomeric region (B).

Table 2
Chemical shifts (ppm) of ^{1}H and ^{13}C NMR signals for the acids-hydrolyzed LHEPS-2, recorded in D_2O at 323 K.

Residue		H-1	H-2	H-3	H-4	H-5	H-6
A	→6-D-Man*p*-(1→	5.46	4.20	4.28	4.11	4.07	3.98
B	→4-D-Gal*p*-(1→	5.33	4.23	4.25	4.13	4.08	3.97
C	T-D-Glc*p*-(1→	5.29	4.09	4.21	3.86	3.96	3.87
D	→4,6-D-Man*p*-(1→	5.26	4.09	4.19	3.96	3.81	3.84
E	→4-D-Man*p*-(1→	5.24	4.09	4.27	4.01	4.08	3.91
F	→6-D-Glc*p*-(1→	5.23	4.09	4.39	4.24	4.10	3.98
		C-1	C-2	C-3	C-4	C-5	C-6
A	→6-D-Man*p*-(1→	102.75	80.59	72.44	72.48	75.51	80.88
B	→4-D-Gal*p*-(1→	104.28	72.27	80.11	72.58	75.51	63.19
C	T-D-Glc*p*-(1→	100.51	80.88	75.51	75.51	69.11	63.30
D	→4,6-D-Man*p*-(1→	100.46	80.88	75.27	75.27	68.93	71.83
E	→4-D-Man*p*-(1→	104.37	72.20	69.11	72.20	68.64	63.30
F	→6-D-Glc*p*-(1→	104.37	71.83	67.86	68.93	69.33	71.83

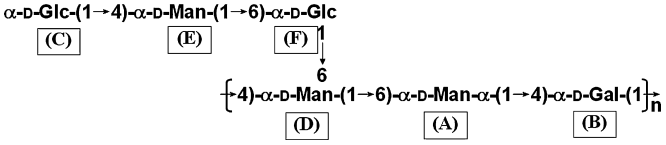

Fig. 6. Proposed structure of LHEPS-2 from *L. helveticus* MB2-1.

4. Conclusion

In the present study, the inhibition of three purified LHEPS fractions (LHEPS-1, LHEPS-2 and LHEPS-3) and crude LHEPS from the *L. helveticus* MB2-1 to the proliferation potential of human gastric cancer BGC-823 cells were studied. The results suggested the potential prospects of LHEPS, especially LHEPS-2 as functional ingredient to prevent gastric cancer. Furthermore, we elucidated the fine structure of the purified LHEPS-2, which was a →4,6-D-Man*p*-(1→6)-D-Man*p*-(1→4)-D-Gal*p*-(1→ backbone with a single T-D-Glc*p*-(1→4)-D-Man*p*-(1→6)-D-Glc*p*-(1→ side-branching in O-6 of →4,6-D-Man*p*-(1→ residue. This structural elucidation was, at least partly, useful for revealing the precise mechanisms underlying the biological effects of LHEPS-2 and in the promotion of food-based therapies. Furthermore, this structural information can be used to gain an insight into the molecular mechanisms involved in heteropolysaccharides-induced anticancer activity. Further work should be performed using *in vivo* models to better understand the potential applications of LHEPS-2 in the treatment of gastric cancer.

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