Carbohydrate Polymers 82 (2010) 895–903

Contents lists available at ScienceDirect

Carbohydrate Polymers

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

Physical characterization of exopolysaccharide produced by *Lactobacillus plantarum* KF5 isolated from Tibet Kefir

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ARTICLE INFO

Article history:
Received 16 April 2010
Received in revised form 29 May 2010
Accepted 7 June 2010
Available online 15 June 2010

Keywords:
Physical characterization
Exopolysaccharide
Lactobacillus plantarum KF5

ABSTRACT

An exopolysaccharide producing strain KF5 was isolated from Tibet Kefir grains and identified as *Lactobacillus plantarum* with morphology, physiological, biochemical and 16S rDNA tests. KF5 exopolysaccharides (EPS) was revealed to have the carboxyl, hydroxyl, and amide groups by FT-IR spectroscopy. The GC analysis of KF5 EPS revealed that it was composed of mannose, glucose and galactose in an approximate ratio of 1:4.99:6.90 in nature. The micro-structure of KF5 EPS under different concentration was observed with atomic force microscopy. The SEM images of KF5 EPS showed smooth surfaces, exhibiting compact structure that showed plasticized films characteristics. Its melting point is 86.35 °C, which is lower than commercial gum. A degradation temperature (Td) of 279.59 °C was determined from the TG curve. It suggested a bit higher degradation temperature than locust gum, but lower than xanthan gum. Physical characteristics of KF5 EPS were different from other commercially available gums, which impart KF5 EPS potential applications in food industry.

1. Introduction

The gradually increasing demand of natural polymers for various industrial applications has led to exopolysaccharide development in recent years, which produced by microorganisms. Many microorganisms can synthesize extracellular polysaccharides and excrete them out of cell. These biopolymers generally include capsular and slime exopolysaccharides (EPS) (Sutherland, 1972). Bacteria and microalgae have a better excreting ability than yeasts and fungi (Sutherland, 1972, 1977, 1982, 1985, 1990). Lactic acid bacteria (LAB) are generally regarded as safe (GRAS). Thus the EPS excreted by LAB can be regarded as safe biology polymer and offer an alternative source of microbial polysaccharides for use in the food or other industries. Many researches showed that EPS amounts and properties are greatly dependent on the microorganisms and their culture conditions and media composition (Cerning, Bouillanne, Landon, & Desmazeud, 1992; Kim et al., 2008; Kimmel, Roberts, & Ziegler, 1998; Looijesteijn & Hugenholtz, 1999; Santivarangkna, Higl, & Foerst, 2008; Sutherland, 1998; Vijayendra, Palanivel, Mahadevamma, & Tharanathan, 2008). Also, EPS, excreted by LAB, of relatively high molecular weight (MW) has been widely studied in the last decade (De Vuyst, De Vin, Vaningelgem, & Degeest, 2001; Laws & Marshall, 2001; Welman & Maddox, 2003). These EPS have special physiochemical and rheological properties, such as viscosifying, stabilizing, gelling or emulsifying which can make them as potential food additives (Jolly, Vincent, Duboc, & Neeser, 2002; Kandler & Kunath, 1983; Sutherland, 1998).

Kefir grains, the starter for obtaining the sour fermented milk kefir, are gelatinous irregular masses, composed of proteins and polysaccharides that contain LAB, acetic acid bacteria and yeasts involved in the fermentation (Abraham & De Antoni, 1999; Garrote, Abraham, & De Antoni, 2001). Kefir grains could produce an exopolysaccharide called kefiran (Kooiman, 1968) and *L. plantarum* KF5 was isolated from Tibet kefir, China. Although there are many reports about EPS produced by *L. plantarum* (Desai, Akolkar, Badhe, Tambe, & Lele, 2006; Mostafa et al., 2006; Nagata et al., 2009; Tsuda, Hara, & Miyamoto, 2008; Sanni, Onilude, Ogunbanwo, Fadahunsi, & Afolabi, 2002), there is dearth of information on physical characteristics of EPS produced by *L. plantarum*.

In the current work, we aimed to preliminary analysis the physical characterization of the EPS produced by strain KF5. Indeed, in a previous work we showed that strain KF5 had the ability of cholesterol-reducing (Zhang, Xu, Xi, & Wang, 2009). Furthermore, *L. plantarum* KF5 has the capability of synthesis EPS. Therefore, the physicochemical characteristics of EPS synthesized by *L. plantarum* KF5 was analyzed to establish a preliminary identification of this biopolymer based on its functional group, monosaccharide composition and physical properties. These characterizations could help us to identify potential application for dairy or other industry and

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doi:10.1016/j.carbpol.2010.06.013

provide some fundamental basis for correlating the intrinsic characteristics of EPS with its putative health benefits in future.

2. Experimental

2.1. Kefir grain

Kefir grain was taken from Tibet, China and was stored in our laboratory and propagated at 25 °C.

2.2. Screening of the isolates for EPS production and media used

Initially *L. plantarum* KF5 and other strains were grown in 50 mL liquid whey medium to screen the strains for EPS quantification. After incubation for 24–48 h, the broth was centrifuged at 10,000 rpm at 4 °C for 15 min. After removing cell, the supernatant was dialyzed and EPS amount was determined by phenol sulphuric acid method using glucose as standard (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956). Results were expressed in mg equivalent of glucose per liter of growth medium. Strain with higher amount EPS productivity was screened out. Whey medium was prepared as described by Yokoi, Watanabe, Fujii, Toba, & Adachi (1990), with some modification. Supplemented whey medium contained (per 100 mL): 1 g lactose monohydrate, 0.5 g glucose, 0.5 g tryptone, 0.05 g cysteine monohydrochloride, 0.5 g sodiumacetate, 0.1 mL Tween 80, 1 mL mineral solution, and 2 g agar. Whey used in the liquid whey media was deproteinized by adjusting whey solution to pH 5.5 with 6 N HCl, heated for 30 min at l00 °C, and centrifuged at 10,000 rpm for 15 min. The supernatant was adjusted to pH 6.8 with 6 N NaOH, heated for 30 min at l00 °C, and centrifuged as mentioned above to obtain deprotenized whey. The mineral solution was composed of 0.4 g/L of $MgSO_4{\cdot}7H_2O$, 0.15 of g/L $MnSO_4{\cdot}4H_2O$, 0.18 g/L of $FeSO_4{\cdot}7H_2O$, and 0.1 g/L of NaCl. The final pH of the whey media was adjusted to 6.0 and was subsequently autoclaved at 115 °C for 20 min. Then the media was inoculated 2% strains (v/v) and incubated at 30 °C for 24 h in an anaerobic incubator with a GasPack filled with gas mixture consisting of 80% N_2, 10% CO_2 and 10% H_2 (v/v).

2.3. Identification of strain KF5

The strain KF5 was primarily identified by Gram's staining and catalase tests. Characteristics of strain KF5 was further identified by morphological, biophysical and biochemical tests. The strain identification was also confirmed by partially sequencing 16S rDNA genes analysis. A primer pair, P1 (5′-AGAGTTTGATCCTGGCTCAG-3′) and P2 (5′-AAGGAGGTGATCCAGCC-3′), corresponding to positions 8–28 and 542–549 of the 16S rDNA respectively, was used to amplify 16S rDNA genes of target isolate. Total chromosomal DNA was extracted as described by Forsman and Alatossava (1994). DNA fragments were amplified as follows: initial denaturation at 94 °C for 4 min, followed by 30 cycles consisting of denaturation at 94 °C for 30 s, annealing at 55 °C for 30 s, extension at 72 °C for 1.5 min and a 10-min final extension step at 72 °C (Zhang, 2003). PCR product was checked with 0.8% agarose gel electrophoresis. Purification of amplified products and verification of the nucleotide sequence were finished by Beijing AuGCT biotechnology Co., ltd. The nucleotide sequences were compared with standard strains for the sequence similarity through BLAST (http://www.ncbi.nlm.nih.gov/blast). Moreover, percentage of sequence similarity was calculated.

2.4. Isolation and purification of EPS

LAB strain KF5 was inoculated into 300 mL optimized fermentation medium and grown at 30 °C for 32 h in anaerobic incubator with a GasPack filled with gas mixture consisting of 80% N_2, 10% CO_2 and 10% H_2 (v/v). The culture was heated for 30 min at 100 °C to dissolve cell attached EPS and then centrifuged at 10,000 rpm for 20 min to separate the cells. Exopolysaccharide in the supernatant fluid was precipitated by adding three times volume of chilled 95% ethanol (−20 °C) and put at 4 °C for 36 h. The sample was then centrifuged at above given conditions and the pellet was retained. The sample was redissolved in distilled water and the chilled absolute ethanol precipitation and water dissolution treatment was repeated twice. The resulting EPS pellet was redissloved in distilled water and then encase in dialysis bag at 4 °C, against four changes of distilled water per day, to remove the small neutral sugars until no momo sugar could be detected outside. The solution in dialysis bag was freeze-dried and further purified by dissolved in 8% trichloroacetic acid (TCA) and stirred over night. Then the deposited protein was shifted by centrifugation at 12,000 rpm for 20 min. The resulting supernatant was adjusted to pH 7.0 and EPS was precipitated by adding an equal volume of chilled ethanol at −20 °C. The pellet was dissolved in double distilled water and was lyphophilized.

2.5. Fourier transform-infrared (FT-IR) spectroscopy

The FT-IR spectrum of purified EPS was detected using Fourier transformed-infrared spectroscopy. For FT-IR spectrum, KF5 EPS was treated using KBr method. The pellets for FT-IR analysis were obtained by grinding a mixture of 1.2 mg of exopolysaccharide with 150 mg of dry KBr powder, followed by pressing the mixture into a mold. The Fourier transform-infrared spectra were recorded on a Bruker Vector 22 instrument (Germany) in the region of 4000–400 cm^{-1}, at a resolution of 4 cm^{-1} and processed by Bruker OPUS software.

2.6. Sugar composition analysis

For sugar composition determinations, polysaccharides were hydrolyzed with 2 M trifluoroacetic acid (TFA) at 120 °C for 3 h. After hydrolysis, water and TFA in the sample were removed by decompressing evaporation. The methanol was added into the dry sample and evaporated by decompression. This treatment repeated five times to remove the residual TFA. The released sugars were converted to their alditol acetates and dissolved in chloroform. And then the samples were filtered through "Supor" membrane (0.22 μm) and used for identification and quantification of the monosaccharide by GC. The standard alditol acetates were generated and analyzed as described by Kang and Qu (2006).

2.7. Atomic force micrograph (AFM) of KF5 EPS

KF5 EPS solution (1 mg/mL) was prepared with distilled water. The aqueous solution was stirred for about 1 h at 40 °C in a sealed bottle under N_2 stream so that KF5 EPS dissolved completely. After cooling to room temperature, the solution was continuously diluted to the final concentration of 0.1 mg/mL, 0.01 mg/mL. About 5 μL of diluted EPS solution was dropped on the surface of a mica sample carrier. And then absolute ethanol was drip on the sample to fix the EPS. The mica carrier was scoured to remove the unabsorbed residue by double distilled water and subsequently allowed to dry at room temperature. Later, the AFM images were obtained by scanning probe microscope (JEOL JSPM-5200, Japan) in tapping mode. The cantilever oscillated at its proper frequency (158 kHz) and the driven amplitude was 0.430 V.

2.8. Scanning electron microscopy (SEM) analysis of KF5 EPS

The microstructure and surface morphology of the copolymers was investigated by scanning electron microscopy (SEM, JEOL/EO,

and model JSM-6380, Japan) at an accelerating voltage of 10 kV. Samples for SEM analysis were glued onto aluminum stubs and gold-sputtered before SEM examination.

2.9. Thermogram analysis (TGA)

The pyrolysis and combustion were carried out in Mettler Toledo TGA/SDTA 851e thermal analyzer operating at atmospheric pressure. The system was controlled by a compatible PC, which registers the temperature measured by a thermocouple placed in the crucible. The crucible was made of Al_2O_3. The 10 mg EPS was placed in a platinum crucible and heated at a linear heating rate of 10 °C/min over a temperature range 25–1000 °C. The experiments were performed separately in air and nitrogen atmosphere at a flow rate of 50 mL/min. Prior to the experiment, TGA/SDTA unit was calibrated for temperature reading using indium as melting standard.

2.10. Analysis of thermal properties

The thermal properties of EPS were analyzed by using a differential scanning calorimeter (DSC Model 141 SETARAM Scientific & Industrial Equipment Co Ltd., France). The 4.2 mg of dried EPS sample was placed in an aluminum pan. Then it was sealed and analyzed, using empty pan as a reference, for determining the melting point and enthalpy change. The heating rate was 10 °C/min from 20 to 300 °C.

3. Results and discussion

3.1. Screening and identification of KF5 strain

Kefir samples were taken from Tibet, China. Different media were used for measuring the amount of EPS produced by different strains, such as supplemented MRS, milk media and supplemented whey media. As a result, we found the supplemented whey media as the best both for screening and exopolysaccharide production. Initially the strains were screened on the basis of the morphology and colonies which have milky, smooth or ropy appearance. In the next step capability of strains to produce EPS was tested by phenol–sulphuric acid method. The strain KF5, which produced higher amount of EPS among screened strains, was selected for present study. Strain KF5 was, Gram positive, catalase negative, nitrate reduction and gelatine liquefaction negative. This strain is brevibacterium (Fig. 1), which can acidify and curd. This homofermentative profile along with the combination of sugar fermentation pattern suggests that strain KF5 might belong to the *L. plantarum* species (Buchanan & Gibbons, 1999; Du, 1992; Ling & Dong, 1999). For further confirmation, partial sequencing of variable regions of 16S rDNA genes was performed. About 1500 base pair (bp) variable regions of 16S rDNA genes was amplified and 1489 bp were sequenced and the Genebank access number was FJ557107. The nucleotide sequences were used for the analysis of sequence similarity through BLAST (http://www.ncbi.nlm.nih.gov/blast) and it gave 100% similarity with *L. plantarum* EW-p, *L. plantarum* NRIC 1834, *L. plantarum* NRIC 1767, *L. plantarum* NRIC 1724, *L. plantarum* NRIC 0387, *L. plantarum* NRIC 0386, *L. plantarum* NRIC 0385, *L. plantarum* NRIC 0384, *L. plantarum* L3, *L. plantarum* WCFS1, *L. plantarum* WCFS1. So strain KF5 was identified and named as *L. plantarum* KF5.

3.2. EPS production, isolation and quantification

Initially *L. plantarum* KF5 produced the superior EPS amount than other strains. So the KF5 strain was chosen for further studying. The yield of strain KF5 was determined as 75.57 mg/L under initial incubation conditions. Effect of different kinds of fermentation conditions, such as incubation time, inoculated amount, and initial pH of culture medium on EPS production was studied in order to enhance EPS production. The optimized incubation conditions were culture for 30 h, initial pH at 6.3, inoculation concentration of 3%, the amount of EPS produced by KF5 could be up to 95.58 mg/L, which increased 26.48% than that under initial fermenting conditions. Many researchers had reported that there are significant relationships among medium composition, culture conditions and pH (Kim et al., 2008; Santivarangkna et al., 2008). The amount of EPS produced by *L. plantarum* and *L. paraplantarum* strains ranged from 140 to 297 mg/L cultivated in MRS broth containing maltose (Zotta, Piraino, Parente, Salzano, & Ricciardi, 2008).

Heating treatment of the samples as the first step in the polysaccharide isolation procedure is crucial for completing recovery of the EPS. Samples without this step gave lower polysaccharide extraction than those including this treatment. But it should be used only where the exopolysaccharide is thermally stable (Kumar, Mody, & Jha, 2007; Rimada & Abraham, 2003).

3.3. Fourier transform-infrared (FT-IR) spectroscopy analysis

Fourier transform-infrared spectroscopy has been a useful tool in monitoring structural changes in biopolymers (Wilson, Goodfellow, & Belton, 1998). The IR spectrum of the purified KF5 EPS is given in Fig. 2, which shows more complex pattern of peaks from 3000 to 1200 cm^{-1}. Polysaccharides contain a significant number of hydroxyl groups, which exhibit an intense broad stretching peak around 3307 cm^{-1}. The absorption in that region (Fig. 2) has the rounded trait typical of hydroxyl groups (Howe, Ishida, &

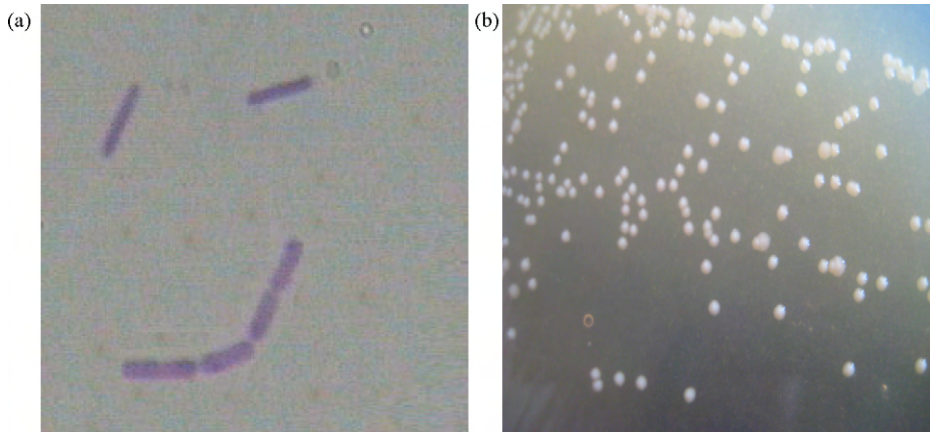

Fig. 1. (a) Micrograph (100×) of strain KF5, and (b) the colonial morphology of strain KF5.

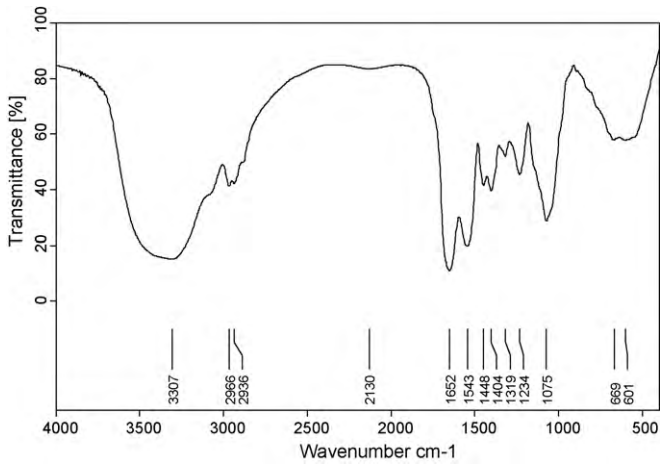

Fig. 2. FT-IR spectrum of the exopolysaccharide produced by *L. plantarum* KF5. The FT-IR spectra were recorded on a Bruker Vector 22 instrument (Germany) in the region of 4000–400 cm^{-1}, at a resolution of 4 cm^{-1} and processed by Bruker OPUS software.

Clark, 2002) which suggests that the substance is polysaccharide. The IR spectra of *L. plantarum* KF5 EPS revealed functional characteristic such as a broad-stretching hydroxyl group at 3307 cm^{-1}, two weak C–H stretching peaks at 2966 and 2936 cm^{-1} corresponding to methyl groups as well as methylene groups. Strong absorption at 1652 cm^{-1} which corresponds to amide I > C=O stretch and C–N bending of protein and peptide amines. And a peak at 1543 cm^{-1} could be assigned to N–H bending of amides II of protein (Lin et al., 2005). The peak at 1448 cm^{-1} was corresponded to asymmetric deformation of CH_3 and CH_2 of proteins. Meanwhile the peak at 1404 cm^{-1} could be assigned to >C=O stretch of the COO^- groups and C–O bond from COO^- groups (Haxaire, Marechal, Milas, & Rinaudo, 2003; Helm & Naumann, 1995). There was no peak around 1700–1775 cm^{-1}, suggesting that neither glucuronic acid nor diacyl ester was present in KF5 EPS, and the peak around 1652 cm^{-1} suggested the presence of the C–O group (Haxaire et al., 2003), which were consistent with results of Wang, et al. (Wang & Bi, 2008). A broad stretch of C–O–C, C–O at 1000–1200 cm^{-1} corresponds to the presence of carbohydrates (Bremer & Geesey, 1991), so in the fingerprint region (region below 1500 cm^{-1} where bands characterize the molecule as a whole), the strongest absorption band at 1075 cm^{-1} is attributed to that substance is polysaccharide (Nataraj, Schomacker, Kraume, Mishra, & Drews, 2008). The FT-IR spectra of the polymer evidenced the presence of carboxyl groups, which may serve as binding sites for divalent cations (Bramhachari et al., 2007). Moreover, the spectrum showed the presence of carboxyl and hydroxyl, which are the preferred groups for the flocculation process similar to that observed in polyelectrolyte (Zajic & Knetting, 1970). Noticeably the exopolysaccharide differ from the EPS produced by *Leuconostoc* sp. CFR 2181 and algal polysaccharide by having an additional peak at around 1240 cm^{-1} region due to the presence of *o*-acetyl ester (Kazy, Sar, Singh, Sen, & Souza, 2004).

3.4. Composition analysis of EPS

The sugar composition of the EPS was analyzed using GC (Fig. 3). The results from present study are given which revealed that KF5 EPS is composed of mannose, glucose and galactose in an approximate ratio of 1:4.99:6.90. Galactose had the highest proportion than mannose or glucose. This result is in accord with some previous reports that in EPS from lactobacilli of food origin, galactose is often found at the same or a higher proportion than the other monosaccharides (Mozzi, Vaningelgem, & Hébert, 2006; Ruas-Madiedo, Salazar, & De los Reyes-Gavilán, 2009). The presence of different sugar moieties suggests that the exopolymer is a heteropolysaccharide. The similar result was reported by Skogen et al. (1974) that *P. zeae* P74 produced an EPS composed of large amounts of mannose and lesser quantities of glucose and galactose. Salazar et al. (2009) reported the composition of EPS were only galactose and glucose (Monosaccharide ration 1:9, 1:5, 1:5, 1:3, respectively), which produced by human origin strains *L. plantarum* C64MRb, E112, G62, H2, respectively. It is well known that in microorganisms, the carbon source used for growth determines both the quality and quantity of polysaccharide formation (Bramhachari et al., 2007). It is reported by Grobben et al. (1997) that the polymer produced by *L. bulgaricus* strain NCFB 2772, grown in chemically defined media containing glucose and fructose, produced two EPS fractions, composed of galactose, glucose and rhamnose. The EPS produced by *Lactobacillus kefiranofaciens* ZW3 was only composed of glucose and galactose (Wang, Zaheer, Feng, Li, & Song, 2008). And Marshall et al. (1995) reported the production of a phosphopolysaccharide by *Lactococcus lactis* subsp. *cremoris* LC330, which consisted of glucose, rhamnose, galactose and glucosamine in an approximate ratio of 6:5:4:1, respectively. Vijayendra et al. (2008) reported the exopolysaccharide produced by a non-ropy strain of *Leuconostoc* sp. CFR 2181 was composed of glucose (91%), rhamnose and arabinose (1.8% each).

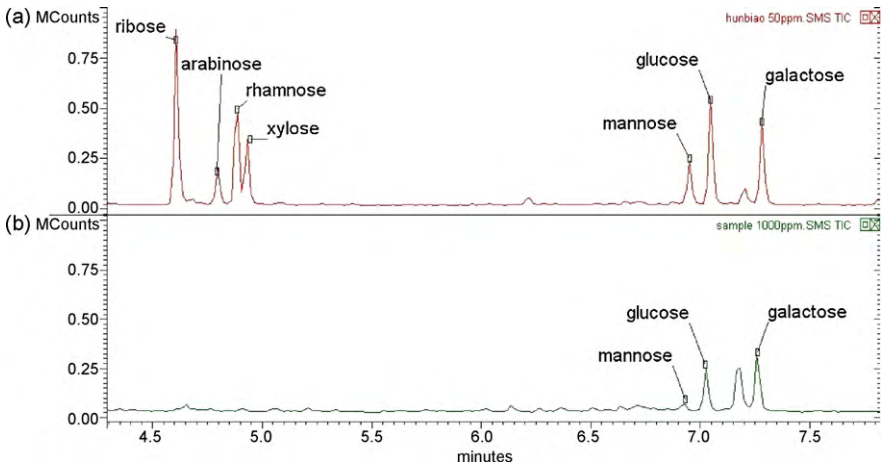

Fig. 3. GC chromatography of alditol acetate derivative of (a) standard monosaccharide and (b) hydrolyzed exopolysaccharide from *L. plantarum* KF5. Polysaccharides were hydrolyzed with 2 M trifluoroacetic acid (TFA) at 120 °C for 3 h. After hydrolysis, water and TFA in the sample were removed by decompressing evaporation. The methanol was added into the dry sample and evaporated by decompression. This treatment repeated five times to remove the residual TFA.

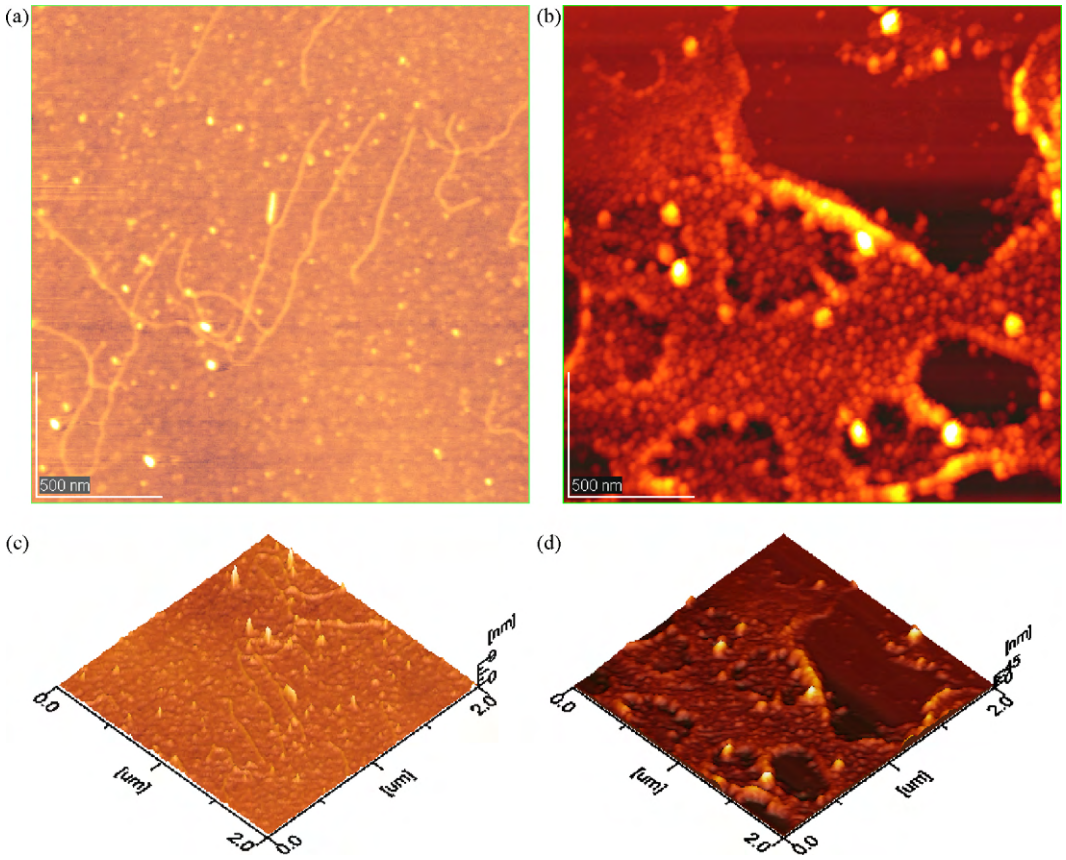

Fig. 4. Atomic force microscopy (AFM) planar (a and b) and cubic (c and d) images of molecular structure of KF5 EPS. The concentration were 10 μg/mL (a and c) and 100 μg/mL (b and d). The atomic force microscopy was a JEOL JSPM-5200 instrument and was operated in the tapping-mode. The cantilever oscillated at its proper frequency (158 kHz) and the driven amplitude was 0.430 V.

3.5. AFM analysis

The AFM-based single-molecule force spectroscopy (AFM–SMFS) technology is a powerful tool to characterize the force-induced conformational transitions, the dynamics, and super molecular structures of polysaccharides at the molecular level (Abu-lail & Camesano, 2003; Giannotti, Rinaudo, & Vancso, 2007; Giannotti & Vancso, 2007; Sletmoen, Maurstad, Sikorski, Paulsen, & Stokke, 2003; Zhang & Marszalek, 2006). AFM imaging of biopolymer as polysaccharides was generally conducted in air or under a liquid in order to avoid excessive dehydration. The typical sample preparation procedure consisted of spreading of a dilute (1–10 μg/mL) polymer solution onto a freshly cleaved mica surface and successive air-drying under ambient pressure, temperature, and humidity (Feng, Gu, Jin, & Zhuang, 2008). The conformation of polymers is thus detected under various environmentally controlled conditions, such as solvent, temperature, salt, and electrochemical potential (Haxaire et al., 2003). The topographical AFM images of KF5 EPS were shown in Fig. 4. KF5 EPS deposited from 10 μg/mL aqueous solution, roundness lumps

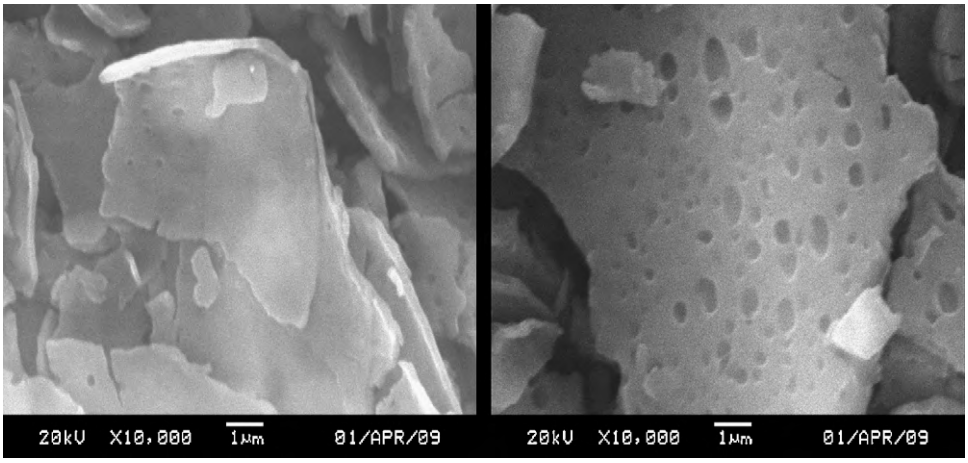

Fig. 5. The microstructure and surface morphology micrographs of KF5 EPS observed by SEM. Magnification is indicated in the micrographs. Samples for SEM analysis were glued onto aluminum stubs and gold-sputtered and analyzed by scanning electron microscopy (SEM, JEOL/EO, and model JSM-6380, Japan) at an accelerating voltage of 10 kV.

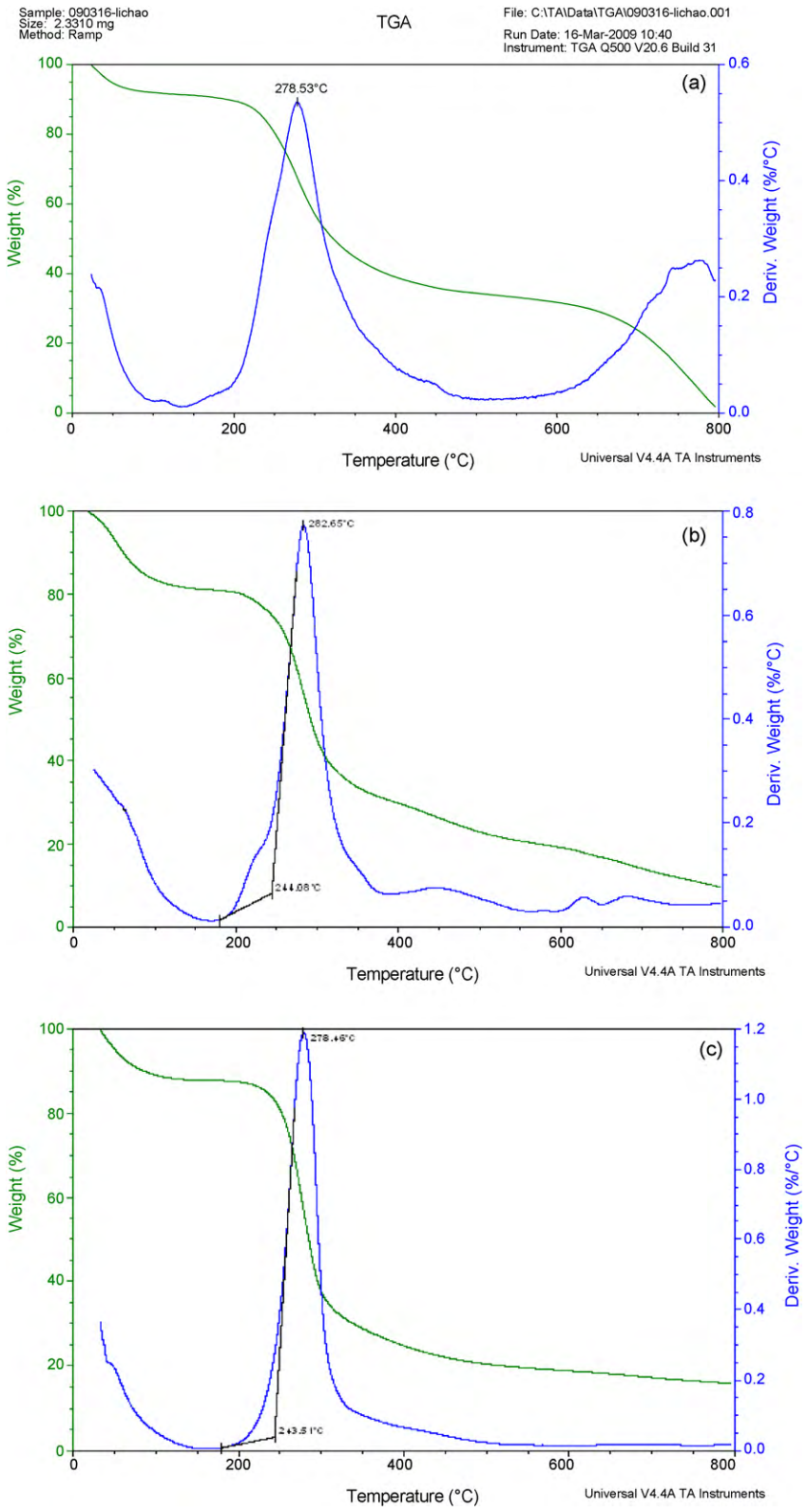

Fig. 6. TG curves of KF5 EPS, xanthan gum, locust gum. (a) KF5 EPS, (b) xanthan gum, (c) locust gum. The pyrolysis and combustion were carried out in Mettler Toledo TGA/SDTA 851e thermal analyzer with compatible PC operating at atmospheric pressure. The 10 mg EPS was placed in a platinum crucible and heated at a linear heating rate of 10 °C/min over a temperature range 25–1000 °C. The experiments were performed separately in air and nitrogen atmosphere at a flow rate of 50 mL/min. Prior to the experiment, TGA/SDTA unit was calibrated for temperature reading using indium as melting standard.

and chains can be seen (Fig. 4a and c). The maximal height of lumps is 13 nm. But the lumps and chains formed at an irregularly reticulation shape when the deposition was made at a higher concentration (100 μg/mL). This result suggested that KF5 EPS could combine water in the aqueous. Furthermore, it showed pseudoplastic behavior because the strong interaction between water molecules and the hydroxyl groups (−OH) of KF5 EPS. A similar experiment was reported about an acidic polysaccharide

from *Mesona blumes* gum (Feng et al., 2008). The AFM images of *Mesona blumes* gum showed different shapes, spherical lumps and worm, respectively in low and high concentration. The reduction in viscosity could also be attributed to polymer degradation due to the cleavage of glycosidic bonds with in the polysaccharide structure (Ren, Ellis, Sutherland, & Ross-Murphy, 2003). These results showed a potential choice for KF5 EPS to be selected as biothickener and stabilizer agent. And strain KF5 can be chosen as the starter to overcome the problem of syneresis in yoghurt industry.

3.6. SEM analysis

Scanning electron microscopy is a very powerful tool to study surface morphology of macromolecules which helps to know about its common physical properties. The microstructure and surface morphology micrographs of KF5 EPS are shown in Fig. 5. As observed by SEM, KF5 EPS resembled sheets of polysaccharide overlaid, appeared as smooth surfaces under 10,000× magnification, exhibiting compact structure which is characteristic of material used to make the plasticized films. So it is the potential chance for making such kind of films.

3.7. Thermogravimetric analysis (TGA)

The thermogravimetric analysis (TGA) of KF5 EPS was carried out dynamically (weight loss versus temperature) and the experimental results are presented in Fig. 6a. A degradation temperature (Td) of 279.59 °C was measured from the TGA curve for KF5 EPS. The polymer showed an initial weight loss between 40 and 100 °C owing to a loss in moisture content. The initial moisture content in the sample is due to the increased level of carboxyl groups in the polysaccharide. This was attributed to the direct positive relationship between the carboxyl contents and the affinity of the polysaccharide for interaction with water (Parikh & Madamwar, 2006). The polymer breaks down above 100 °C and the weight was lost. The weight of polymer dramatically lost around 300 °C and gradually decreased. Fig. 6b and c shows the TG analysis of xanthan gum and locust gum respectively as reference material. Degradation temperature for xanthan gum is 282.65 °C, where for locust gum is 278.46 °C. Thus the EPS of KF5 showed a bit higher degradation temperature than locust gum. These results may due to the different structure and composition among KF5 EPS, locust gum and xanthan gum. The gum of the locust bean (LBG), *Ceratonia siliqua*, is derived from the endosperm of the seeds after removal of the testa (seed coat), and the quality of the gum is dependent on the degree of separation achieved (KÖk, Hill, & Mitchell, 1999). Locust gum is a linear polysaccharides, which is composed of mannose and galactose. The average mannose to galactose ratio (M/G) in LBG is approximately 3.5:1 (Dakia, Blecker, Robert, Wathelet, & Paquot, 2008). Xanthan gum is an extracellular hetero-polysaccharide, which is produced by the aerobic fermentation of Xanthomonas campestris. Xanthan is composed of pentasaccharide repeating units, containing D-glucose, D-mannose, D-glucoronic acid (at a ratio 2:2:1), acetal-linked pyruvic acid and D-acetyl groups (Baird, 1989). However, KF5 EPS is an extracellular heteropolysaccharide, which produced by *L. plantarum* KF5. The composition of KF5 EPS was mannose, glucose and galactose in an approximate ratio of 1:4.99:6.90.

3.8. Analysis of thermal properties

Besides chemical properties, applicability of polysaccharide is largely dependent on its thermal behaviour (Marinho-Soriano & Bourret, 2005). As for the thermal characteristics of exopolysaccharides, heat absorption and emission are accompanied with the physical change by deformation of polymer structure or melting of crystalline polysaccharides. Energy level of the polysaccharide was scanned using a differential scanning calorimeter and was compared with xanthan gum, guar gum and locust gum used as standard. The melting temperature of KF5 EPS, xanthan gum, guar gum and locust gum started at about 86.35, 153.4, 490.1 and 109.11 °C, respectively, and the endothermic enthalpy change (ΔH) required to melt 1 g of KF5 EPS, xanthan gum, guar gum and locust gum were 133.5, 93.2, 192.9 and 87.1 J, respectively (Table 1). The KF5 EPS suggest the lowest melting points than other commercial gum and the enthalpy only lower than guar gum. Thus the KF5 polysaccharide showed a different thermal behavior from xanthan gum, guar gum and locust gum. As for the earlier report that exopolysaccharides obtained from a mutant of *Bacillus polymyxa*, the melting point was 183.25 °C, and enthalpy was 100.3 cal/g (Kwon, Joo, & Oh, 1992). And the measurement of the thermal characteristics of levan synthesized with levansucrase showed the highest melting point to be 178.4 °C with an enthalpy of 1.66 cal/g, which was similar to the thermal characteristics of the exopolysaccharides derived from legacy microorganisms (Jung, Song, Kim, Chun, & Rhee, 1999).

Table 1
Thermal properties of KF5 EPS, xanthan gum, guar gum and locust gum determined by differential scanning calorimetry (DSC).

Sample name	Peak temperature (°C)	Enthalpy (J g^{-1})
KF5 EPS	86.35	133.5
Xanthan gum	153.4	93.2
Guar gum	490.1	192.9
Locust gum	109.11	87.1

4. Conclusion

The research on microbial exopolysaccharides is attracting increased attention. In this study, the EPS producing strains *L. plantarum* KF5 was identified, which also had cholesterol-reducing ability (Zhang et al., 2009). The KF5 EPS was a typical heteropolymeric polysaccharide, which composed of mannose, glucose and galactose in an approximate ratio of 1:4.99:6.90 in nature. It suggested from the SEM and AFM images that KF5 EPS has potential ability to make the biofilm. In addition, exopolymer showed a lower melting point and a higher degradation temperature. These characteristic of KF5 EPS enhanced its potential uses, especially for food industry. Further work is needed to investigate the applications and function in vitro for this EPS.

Acknowledgments

This work was supported by Tianjin Municipal Science and Technology Commission Grant (No. 08JCYBJC01900) and The Research Fund for the Doctoral Program of Higher Education (No. 20091208110001).

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