Food Hydrocolloids 30 (2013) 343–350

Contents lists available at SciVerse ScienceDirect

Food Hydrocolloids

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

Characterization of exopolysaccharide produced by *Lactobacillus kefiranofaciens* ZW3 isolated from Tibet kefir – Part II

Zaheer Ahmed [a,b,*], Yanping Wang [b], Nomana Anjum [a], Asif Ahmad [c], Salman Tariq Khan [d]

[a] Department of Home & Health Sciences, Allama Iqbal Open University, Islamabad, Pakistan
[b] Tianjin Key Laboratory of Food Nutrition and Safety, Faculty of Food Engineering and Biotechnology, Tianjin University of Science and Technology, People's Republic of China
[c] Department of Food Technology, Pir Mehr Ali Shah Arid Agriculture University Rawalpindi, Pakistan
[d] Pharmaceutical Research Centre, PCSIR Labs Complex, Karachi 75280, Pakistan

ARTICLE INFO

Article history:
Received 16 October 2011
Accepted 6 June 2012

Keywords:
Lactobacillus kefiranofaciens ZW3
Rheological
Tibet kefir
Characterization

ABSTRACT

ZW3 is a newly discovered exopolysaccharide (EPS) produced by *Lactobacillus kefiranofaciens* ZW3, isolated from Tibet kefir. Some of its properties have been characterized in our previous paper. Present research demonstrates some other important aspects of this EPS. The molecular weight obtained by gel permeation HPLC was 5.5×10^4 Da. Solubility, water holding and oil binding capacity of ZW3 EPS were 14.2%, 496.0%, and 884.74% respectively. Scanning electron microscopy (SEM) of ZW3 EPS demonstrated a smooth surface with compact structures. A topographical examination of EPS by atomic force microscopy (AFM) revealed that ZW3 EPS is composed of almost uniform net of molecules. Rheological study indicated that common salt did not affect the viscous behavior of ZW3 EPS and acidic pH may enhance its viscosity. Exopolymer showed a melting point of 93.38 °C. A degradation temperature (Td) of 299.62 °C was observed from the TGA curve for the polysaccharide ZW3.

1. Introduction

In recent years, polysaccharides have attained the considerable attention of researchers because of their wide distribution in nature and documented health benefits. These macromolecules are either homopolymers or heteropolymers of neutral sugars (Badel, Bernardi, & Michaud, 2011). The thickening properties of polysaccharides make them ideal as food additives and can be extracted through various sources including plant, fungi or seaweeds (Saija, Welman, & Bennett, 2010). Extraction and purification processes may affect the physiochemical and structural properties of polysaccharides and hence characterization of polysaccharides is essential to determine suitable properties for the purpose of their utilization as food additives. Often the structure of these polymers is modified to increase their rheological properties and to make them suitable for various food applications (De Vuyst & Degeest, 1999; Roller & Dea, 1992). Another reason for acceptance of lactic acid bacterial polysaccharides such as ZW3 EPS is that, addition of certain polysaccharides from plant sources is not acceptable in certain dairy products due to problem of "all dairy" label to dairy foods; and also the use of such plant polymers in dairy products is prohibited in many European countries (Saija et al., 2010; Wang, Zaheer, Feng, Li, & Song, 2008). These restrictions force the agro-food industries to look for other possible sources of polymers from dairy sources and for that the best option is exopolysaccharide produced by lactic acid bacteria on dairy based source with a GRAS (generally regarded as safe) status (Badel et al., 2011; Maeda, Zhu, Suzuki, Suzuki, & Kitamura, 2004; Wang et al., 2008, 2010).

Numerous strains of lactobacillus genera have a potential to produce exopolysaccharide under specific growth conditions with a wide range and diversity of structure and have a potential to be used as nutraceuticals (Badel et al., 2011; Gorska et al., 2010; Wang et al., 2008, 2010). Due to their characteristic functional properties, LAB exopolysaccharides are used as stabilizing, viscosity modifying, and gelling agents (Pan & Mei, 2010). However the physiological function of these polymers is still unknown and few are used in food industries (Suresh Kumar, Mody, & Jha, 2007; Sutherland, 2007).

Lactobacillus kefiranofaciens, an isolate from kefir is famous for its polymer named as kefiran; and has attained the attention of many researchers in recent years (Piermaria, de la Canal, & Abraham, 2008, Piermaria, Pinotti, García, & Abraham, 2009; Wang & Bi, 2008; Wang et al., 2008). Deproteinized whey medium which is waste product of cheese industry can be used as substrate which ends in the production of a valuable product i.e. kefiran (Wang et al., 2008). These carbohydrates have several health beneficial effects, including the decrease of blood pressure induced by hypertension

* Corresponding author. Department of Home & Health Sciences, Allama Iqbal Open University, Islamabad, Pakistan. Tel.: +92 519057265; fax: +92 51 9250063.
E-mail address: zaheer_863@yahoo.com (Z. Ahmed).

0268-005X/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.foodhyd.2012.06.009

(Maeda et al., 2004), immunomodulation, epithelium protection and antagonic activity against *Bacillus cereus* on Caco-2 cells (Piermaria et al., 2010), increased phagocytic activity of peritoneal and lung macrophages (Vinderola, Perdigon, Duarte, Farnworth, & Matar, 2006) and increased IgA cells in these sites (Duarte, Vinderola, Ritz, Perdigon, & Matar, 2006), antitumor activity (Liu, Wang, Lin, & Lin, 2002), antimicrobial activity (Rodrigues, Caputo, Carvalho, Evangelista, & Schneedorf, 2005), and anti-inflammatory activity (Moreira et al., 2008). Kefiran also improves the rheological properties and better viscoelasticity can be achieved through addition of kefiran up to a level of 300 mg/L (Badel et al., 2011; Piermaria et al., 2010). Moreover, the kefiran can form brittle and transparent films with good water vapor barrier characteristics (Piermaria et al., 2009). Due to beneficial attributes of this polymer the present research was conducted. In our previous paper (Wang et al., 2008) we have characterized some of properties of polymer produced by *L. kefiranofaciens* ZW3 isolated from Tibet kefir. The strain produces a high amount of polymer having desirable physiochemical properties (Wang et al., 2008). However, to explore its potential for application in food industry more characterization on physiochemical, structural and rheological parameters is required. Keeping in view all of this, the current project was planned to discover industrially important physiochemical, structural and rheological parameters for this exopolysaccharide.

2. Materials and methods

2.1. Isolation and purification of EPS

The indigenous strain *L. kefiranofaciens* ZW3 was isolated and further purified from Tibet kefir as described in our previous study (Wang et al., 2008). Liquid whey media was used for its propagation and media was incubated under anaerobic conditions at temperature of 30 °C and for a time period of 72 h. Maximum recovery of EPS was obtained when degrading enzymes were inactivated by heating the media at a temperature of 100 °C for 30 min. This was followed by ultracentrifugation at 12,000 × *g* for 15 min at refrigerated temperature. EPS was precipitated by using the chilled absolute ethanol and was kept in refrigerator at temperature of 4 °C for 12 h, followed by a second centrifugation by maintaining the above parameters. For further purification, EPS obtained by above treatment was redissolved in distilled water (100 ml) with gentle heating below 50 °C and precipitated again with equal volume of chilled absolute ethanol. Again an ultracentrifuge treatment was applied (25,000 × *g*) for 25 min at refrigeration temperature. To achieve further purification, EPS pellets were once again redissolved in 20 ml of distilled water with gentle heating (below 50 °C). Dialysis technique was applied to remove small molecular weight simple sugars at 4 °C for 72 h with three changes of distilled water in a day. Dialyzed EPS was recovered through freeze dryer. Recovered EPS was named as partially purified EPS and was further characterized for some important parameters. Trichloroacetic acid (TCA 14%) was used for further purification by overnight stirring. This technique is valuable to remove protein impurities from EPS. Precipitated protein was separated through centrifugation at 12,000 × *g* for 15 min. The resultant material was neutralized up to pH level of 7.0 and was again precipitated by adding chilled ethanol in equal volumes. Finally pellets of EPS were redissolved in double distilled water and were lyophilized.

2.2. Study of common physical properties

Solubility of ZW3 EPS in water and oil was determined by following the procedure of Chang and Cho (1997). A separate suspension of EPS was made by dissolving EPS at rate 50 mg/ml in water and oil with continuous agitation at 25 °C for 24 h. This was followed by centrifugation 5000 × *g* for 15 min and collected supernatant (0.2 ml) was precipitated with 3 volume of ethanol. Again EPS in form of precipitate was recovered by centrifugation at 10,000 × *g* for 5 min. Resultant material was vacuum dried at 50 °C and difference in weight was recorded .The solubility was calculated as follows:

$$\text{Solubility}(\%) = [\text{Total carbohydrate concentration in supernatant(a)}]/[\text{Weight of sample (dry weight basis)}] \times 100$$

Sample of EPS was characterized for water holding capacity (WHC) by suspending 0.2 g sample in 10 ml of deionized water on a vortex mixer. Dispersed material was centrifuged at 16,000 × *g* for 25 min. Unbound water that was not held by EPS material was discarded. All EPS material was dropped on pre weight filter paper for complete drainage of water. Weight of EPS precipitated was recorded. The percentage of WHC was calculated through following expression:

$$\text{WHC}(\%) = [\text{total sample weight after water absorption}]/[\text{total dry sample weight}] \times 100$$

The oil binding capacity was also calculated for this EPS in a similar manner by adopting method of Kato, Okamoto, Tokuya, and Takahashi (1982). For that purpose soya bean oil was used as dispersing media. The other steps were identical to the analysis procedure of WHC.

2.3. Measurement of molecular weight

Extracted and refined EPS pellet was characterized for molecular weight using Agilent 1100series HPLC system (Agilent technologies Palo AHO, CA, USA). Equipment was equipped with refractive index detector TOSOH TSK-G4000 PWxl column (7.8 mm × 30 cm, 10 μm) (TOSOH Corp., Tokyo, Japan). A sample of 20 μL was injected in the system by maintaining a flow rate of 0.5 ml/min and column temperature of 35 °C. Separation was carried out by using 0.71% sodium sulfate as mobile phase. Dextran D2000 with molecular weight of 2×10^6, D8 with molecular weight of 1.338×10^5, D7 with molecular weight of 41,100, D5 with molecular weight 21,400, D4 with molecular weight 10,000, D0 with molecular weight 180 (glucose) was used as reference compound. These reference standards were added into the mobile phase at rate of 10 mg per 1 ml solution.

2.4. Measurement of rheological properties of ZW3 EPS

Brookfield Digital Rheometer Model DV III (Brookfield Engineering Laboratories Inc., Stoughton, Massachusetts, USA) was used to determine rheological properties. An RV type ULA spindle that rotated in chamber equipped with temperature control system (Thermomixs; B. Braun Biotech International) was attached with rheometer. Brookfield Rheocalc software (Brookfield Engineering Laboratories Inc.) was used to control the instrument.

For preparation of sample EPS was well dissolved at rate of 2 mg/ml and 4 mg/ml. Rheological behavior for test solution was measured against time with increasing shear rate. Further rheological characteristic of EPS was performed at variable pH levels and with addition of different salts. pH of EPS solution was adjusted at level of 4.0, 5.0 and 6.5 by using lactic acid. Two salts solutions; NaCl (0.1 M) and $CaCl_2$ (0.1 M) separately were used to dissolve EPS for characterization of rheological properties. Further characterization was carried out by dissolving EPS in skim milk and water and their rheological properties were compared with each other.