Bioactive Carbohydrates and Dietary Fibre 6 (2015) 55–61

Contents lists available at ScienceDirect

Bioactive Carbohydrates and Dietary Fibre

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

In vitro analysis of dextran from *Leuconostoc mesenteroides* NRRL B-1426 for functional food application

Damini Kothari, Jagan Mohan Rao Tingirikari, Arun Goyal *

Department of Biotechnology, Indian Institute of Technology Guwahati, Guwahati 781039, Assam, India

ARTICLE INFO

Article history:
Received 5 June 2015
Received in revised form
20 August 2015
Accepted 21 August 2015

Keywords:
Dextran
Biocompatible
Leuconostoc mesenteroides
Prebiotic

ABSTRACT

The present study describes physico-chemical properties, prebiotic potential and biocompatibility of dextran produced from *Leuconostoc mesenteroides* NRRL B-1426. Dextran displayed porous surface, pseudoplastic non-Newtonian behavior, 32% solubility, 290% water holding capacity and a degradation temperature of 290 °C. The dextran exhibited significantly higher non digestibility as compared with the standard prebiotic, inulin in simulated human gastric juice and α-amylase. The dextran significantly stimulated the growth of probiotics, bifidobacteria and lactobacilli and was comparable to that by inulin. The viability of human embryonic kidney (HEK 293), human cervical adenocarcinoma (INT-407) and human colon carcinoma (HT-29) cell lines by dextran treatment (50–1000 μg/ml) remained unaffected displaying the biocompatibility of the dextran. Therefore, the dextran from *L. mesenteroides* NRRL B-1426 can act as potential prebiotic, gelling agent and be used as food supplement for health benefits.

1. Introduction

The human colon is one of the most metabolically active and diversely colonized organs with thousands of different bacterial species (approximately 10^{11}–10^{12} cfu/g) (Slavin, 2013). The functional food science, a popular concept, aims to fortify colonic microbiota through the use of prebiotics (Wang, 2009; Saad et el., 2013). Prebiotics are "food components/ingredients/supplements which result in the selective stimulation of growth and/or activity (ies) of one or a limited number of microbial genus(era)/species in the gut microbiota that confer(s) health benefits to the host" (Roberfroid et al., 2010). The established prebiotics include galactooligosaccharides (GOS), fructooligosaccharides (FOS) and inulin (Al-Sheraji et al., 2013). Recently, the dextrans have also shown emerging prebiotic potential (Das et al., 2014; Tingirikari et al. 2014a; Sarbini et al., 2013) besides their viscosifying, texturizing, gelling (Purama et al., 2009), biodegradability or biocompatibility properties (Siddiqui et al., 2014; Patel et al., 2010). In addition, the enzymatic cleavage of dextran by free or immobilized dextranase selectively synthesizes prebiotic oligosaccharides (Bertrand et al., 2014). Dextran is a bacterial homopolymer of D-glucose with α-(1→6) linkage in the main chain and variable amount of α-(1→2), α-(1→3) and α-(1→4) branched linkages (Jeanes et al., 1954). The biological activities of dextran are determined by their glycosidic linkages, monosaccharide composition and degrees of polymerization degrees. (Delattre and Vijayalakshmi, 2009). Polysaccharides with prebiotic effect have several advantages over non digestible oligosaccharides, such as decreased caloric intake, tolerance to high ingestion, mucosal damage from rapid acidification and laxative effect in the colon (Ellegard et al., 1997). In addition, large polysaccharides afford a persistent source of fermentable carbohydrate throughout the colon rather than being completely fermented proximally, which may prevent colon cancer. It was also reported that dextran and oligodextran which consist of α-(1→6) glucosidic backbones are less susceptible to attack by human and animal digestive enzymes (Olano-Martin et al. 2000; Auriol and Monsan, 2004). The branched dextrans were resistant to hydrolysis by exodextranases and glucosidases (Remaud-Simeon et al., 2000). The dextran from *L. mesenteroides* NRRL B-1426 used in the present study contains 85.5% of α-(1→6) linear and 14.5% α-(1→3) branched linkages (Kothari and Goyal, 2013). In this work, the physicochemical properties, prebiotic potential and effect on mammalian cell lines of dextran from *L. mesenteroides* NRRL B-1426 have been investigated.

2. Materials and methods

2.1. Chemicals and reagents

All the media components for maintenance and enzyme production were purchased from Hi-Media Pvt. Ltd., India. Serine, disodium phosphate, bichinconinic acid and α-amylase (from

* Corresponding author. Fax: +91 361 2582249.
E-mail address: arungoyl@iitg.ernet.in (A. Goyal).

http://dx.doi.org/10.1016/j.bcdf.2015.08.001
2212-6198/

human saliva), Dulbecco's Modified Eagle's Medium (DMEM), minimum essential medium (MEM), Roswell Park Memorial Institute (RPMI 1640) medium, fetal bovine serum (FBS), sodium bicarbonate, sodium pyruvate, antibiotic antimycotic solution, trypsin EDTA and 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) were purchased from Sigma Aldrich, USA. All the chemicals used for reducing sugar estimation, protein estimation and buffer preparation were of high purity.

2.2. Microorganisms

L. mesenteroides NRRL B-1426, *Bifidobacterium animalis* sub species *lactis* NRRL B-41405, *Bifidobacterium infantis* NRRL B-41661 and *Lactobacillus acidophilus* NRRL B-4495 were procured from ARS culture collection, National Center for Agricultural Utilization Research, Peoria, USA. *Escherichia coli* DH5α was procured from Novagen.

2.3. Dextran production and purification

Dextran was produced by incubating 1 ml of purified dextransucrase (0.75 mg/ml, 10.1 U/mg) from *L. mesenteroides* NRRL B-1426 in 10 mL of reaction mixture 146 mM sucrose in 20 mM sodium acetate buffer (pH 5.6) containing 0.3 mM $CaCl_2$ and 15 mM NaN_3 at 30 °C for 24 h. The dextran produced was purified by ethanol precipitation as described by Kothari and Goyal (2013), and lyophilized for further analysis.

2.4. Solubility and water holding capacity of dextran

The solubility of dextran in water was determined by the method described by Chang and Cho (1997). 50 mg of dextran was dissolved in 1 ml of deionised water with continuous stirring at 25 °C for 24 h. The dextran suspension was then centrifuged at 5000 g and 25 °C for 15 min. Three volume of ethanol (95%) was added to 0.2 ml collected supernatant and centrifuged again at 10,000 g and 4 °C for 20 min. The precipitate obtained was vacuum dried at 80 °C and difference in weight was measured. The solubility of dextran was calculated as follows:

$$\text{Solubility}(\%) = \frac{\text{Total dextran concentration in supernatant}}{\text{Initial dry weight of dextran}} \times 100$$

Water holding capacity (WHC) of dextran was determined by the method described by Ahmed et al. (2013). 0.2 g of dextran was dissolved in 10 mL of deionised water and was centrifuged at 13,000 g and 4 °C for 30 min. The unbound water not held by dextran was discarded. The precipitated dextran was placed on pre weighed filter paper for complete removal of water. The wet weight of precipitated dextran was determined. The percentage of WHC was calculated as follows:

$$\text{WHC}(\%) = \frac{\text{Wet weight of dextran after water absorption}}{\text{Initial dry weight of dextran}} \times 100$$

2.5. Rheology of dextran solution

The rheological measurement (viscosity vs shear rate) of an aqueous solution of dextran (0.5%, *w*/*v*) was performed at 25 °C using a rheometer (Thermo Electron, Haake ReoStress 1) interfaced with a HAAKE RheoWin 323 software. The applied shear rate was in the range of 10–340 s^{-1}.

2.6. Thermo-gravimetric analysis of dextran

The measurement of thermal stability of dextran was carried out using a differential scanning calorimeter/thermo-gravimetric analyzer (DSC/TGA) (STA 449 F3 *Jupiter,* NETZSCH) with Proteus software. 5 mg of dextran was placed on alumina (Al_2O_3) crucible and heated at a constant rate 10 °C/min over a temperature range 20–600 °C in nitrogen atmosphere (60 ml/min).

2.7. Prebiotic activity assay for dextran

2.7.1. Effect of simulated human gastric juice on digestibility of dextran

Digestibility of dextran was tested by calculating the percent hydrolysis when subjected to simulated human gastric juice according to the method of Korakli et al. (2002). The simulated human gastric juice was prepared using hydrochloric acid buffer containing (g/l) NaCl, 8; KCl, 0.2; $Na_2HPO_4 \cdot 2H_2O$, 8.25; NaH_2PO_4, 14.35; $CaCl_2 \cdot 2H_2O$, 0.1 and $MgCl_2 \cdot 6H_2O$, 0.18. The pH of the buffer was adjusted to 1, 2, 3 and 4 by 5 M HCl. Dextran and inulin samples (1 ml, 1% *w*/*v*) were mixed with 1 mL of simulated human gastric juice at the four different pH and incubated at 37 °C for 6 h. Aliquots (100 μl) of the reaction mixture were collected from each treatment at 0, 0.5, 1, 2, 4 and 6 h intervals to determine the reducing sugar and total sugar. Total sugar (glucose equivalents) and reducing sugar (maltose equivalents) were determined before and after digestion by phenol sulfuric acid and copper-bicinchoninate methods, respectively (Fox and Robyt, 1991). Per cent hydrolysis of samples was calculated by the following equation given by Korakli et al., (2002).

$$\text{Hydrolysis}(\%) = \frac{\text{Reducing sugar released}}{\text{Total sugar} - \text{Initial reducing sugar}} \times 100$$

Where the reducing sugar released is the difference between the final reducing sugar after hydrolysis and the total initial reducing sugar.

2.7.2. Effect of human α-amylase on digestibility of dextran

The effect of human α-amylase on digestibility of dextran from *L. mesenteroides* NRRL B-1426 and inulin was determined by following the method of Wichienchot et al. (2010). A solution of α-amylase (2 U/ml) was prepared in 20 mM sodium phosphate buffer containing 6.7 mM sodium chloride with varying pH 5, 6, 7 and 8. Dextran and inulin samples (1 ml, 1%, *w*/*v*) were mixed with 1 ml of α-amylase solution at the four pH levels separately and the reaction mixtures were incubated at 37 °C for 6 h. Aliquots (100 μl) of the reaction mixture were collected from each treatment at 0, 0.5, 1, 2, 4 and 6 h intervals to calculate the percent hydrolysis as described in the Section 2.7.1.

2.7.3. Effect of dextran and inulin on the growth of human gut bacteria

Two bifidobacterial (*B. animalis* sub species *lactis* and *B. infantis*) and one lactobacilli strains (*L. acidophilus*) were chosen as probiotics, while *E. coli* was used as a potential coliform for prebiotic activity analysis. The log phase cultures (1%, v/v) of *Bifidobacteria* and *Lactobacillus* were inoculated in 10 ml MRS medium (pH 6.4) (De-Man, Rogosa, and Sharpe, 1960), whereas *E. coli* into 10 ml TGY medium (pH 7.0) (Deak et al., 1998). The cultures were treated with 1% (*w*/*v*) of dextran and commercial inulin as carbon source (dextran and inulin were autoclaved separately and was added to the media). The respective growth media (MRS and TGY) without any carbon source were used as negative controls. The bacterial cultures were incubated at 37 °C under anaerobic conditions in anaero bags (Hi-Media Pvt. Ltd., India). The bacterial growth was monitored after 48 h as absorbance at 600 nm (A_{600}) using UV visible Spectrophotometer (Varian, Cary 100) and was correlated with the carbohydrate utilization in the medium. The carbohydrate utilized was estimated by phenol sulfuric acid