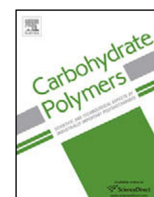
Carbohydrate Polymers 174 (2017) 646–657

Contents lists available at ScienceDirect

Carbohydrate Polymers

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



Rheology and bioactivity of high molecular weight dextrans synthesised by lactic acid bacteria

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ARTICLE INFO

Article history:
Received 31 December 2016
Received in revised form 24 June 2017
Accepted 28 June 2017
Available online 29 June 2017

Keywords:
Exopolysaccharides
Dextran
Lactic acid bacteria
Leuconostoc mesenteroides
Rheological properties
Immunomodulation
Adhesion

ABSTRACT

Dextrans synthesised by three *Leuconostoc mesenteroides* strains, isolated from mammalian milks, were studied and compared with dextrans produced by *Lc. mesenteroides* and *Lactobacillus sakei* strains isolated from meat products. Size exclusion chromatography coupled with multiangle laser light scattering detection analysis demonstrated that the dextrans have molecular masses between 1.74×10^8 Da and 4.41×10^8 Da. Rheological analysis of aqueous solutions of the polymer revealed that all had a pseudoplastic behaviour under shear conditions and a random, and flexible, coil structure. The dextrans showed at shear zero a difference in viscosity, which increased as the concentration increased. Also, the purified dextrans were able to immunomodulate *in vitro* human macrophages, partially counteracting the inflammatory effect of *Escherichia coli* O111:B4 lipopolysaccharide.

During prolonged incubation on a solid medium containing sucrose, dextran-producing bacteria showed two distinct phenotypes not related to the genus or species to which they belonged. Colonies of *Lc. mesenteroides* CM9 from milk and *Lb. sakei* MN1 from meat formed stable and compact mucoid colonies, whereas the colonies of the other three *Leuconostoc* strains became diffuse after 72 h. This differential behaviour was also observed in the ability of the corresponding strains to bind to Caco-2 cells. Strains forming compact mucoid colonies showed a high level of adhesion when grown in the presence of glucose, which decreased in the presence of sucrose (the condition required for dextran synthesis). However no influence of the carbon source was detected for the adhesion ability of the other *Lc. mesenteroides* strains, which showed variable levels of binding to the enterocytes.

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Abbreviations: C*, critical concentration for interaction between dextran molecules; CDMS, CDM define medium lacking glucose and supplemented with 0.8% sucrose; cfu, colony forming unit; CM9, CM30, SM34 and RTF10, *Leuconostoc mesenteroides* strains; DMEM, Dulbecco's Modified Eagle medium; EDTA, ethylenediaminetetraacetic acid; EPS, exopolysaccharide; $\dot{\gamma}$, shear rate; HePS, heteropolysaccharides; HoPS, homopolysaccharides; HPLC, high-performance liquid chromatography; LAB, lactic acid bacteria; LPS, lipopolysaccharide from *Escherichia coli* O111:B4; MRSG, Man Rogosa Sharpe broth containing 2% glucose; MRSS, MRS medium containing 2% sucrose instead of glucose; MSE, Mayeux Sandine Elliker medium; η_0, viscosity at near-zero shear rate; η, apparent viscosity; PBS, phosphate-buffered saline; PMA, phorbol 12-myristate 13-acetate; SEC-MALLS, size exclusion chromatography coupled to multiangle laser light scattering detection; PMA-THP-1, THP-1 monocytes differentiated to macrophages with PMA; TEM, transmission electron microscopy.

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http://dx.doi.org/10.1016/j.carbpol.2017.06.113
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1. Introduction

Lactic acid bacteria (LAB) are a heterogeneous group of gram-positive bacteria, traditionally used in food fermentation and preservation (Gaspar, Carvalho, Vinga, Santos, & Neves, 2013). Currently, their usage has been expanded to the manufacture of functional food, as efficient microbial cell factories for production of industrially relevant metabolites and as probiotics that impact general health and well-being (Mozzi, Raya, & Vignolo, 2015). The positive contribution of LAB to functional food manufacture is assured by the production of beneficial compounds such as lactic acid, carbon dioxide, aroma compounds, vitamins, antimicrobial agents, polyols and exopolysaccharides (EPS) (Nuraida, 2015). The latter bio-products are synthesised by some LAB and remain associated with the cell surface constituting a capsule, or released as a free polymer into the environment (Nwodo, Green, & Okoh, 2012). The LAB EPS can differ according to their chemical structure, molecular mass and linkage types, which could modulate their biological functionality (Surayot et al., 2014). According to monomeric composition, EPS are classified into two groups: homopolysaccharides (HoPS) contain a single type of monosaccharide and heteropolysaccharides (HePS), which are composed of repeated units of at least two types of monosaccharides and various types of linkages (Patel, Majumder, & Goyal, 2012).

The HoPS can be α-D-glucans, β-D-glucans or β-D-fructans and they are respectively synthesized by glucansucrases, glycosyltransferases or fructansucrases (Pérez-Ramos et al., 2015; Werning et al., 2012). HoPS are able to modulate rheological properties of materials (Welman & Maddox, 2003), and several studies have shown that they can replace or reduce the use of more expensive hydrocolloids and could find applications such as texturizing agents (Bounaix et al., 2009). Dextran is a HoPS composed of a main linear chain of α-1,6 glycosidic linkages with a few branches of α-glucopyranose at positions *O*-2, *O*-3 or *O*-4. This biodegradable polymer is synthesised by LAB mainly belonging to the *Leuconostoc*, *Lactobacillus* and *Streptococcus* genera (Ciszek-Lenda, 2011; Patel et al., 2012). Its yield is affected by environmental conditions, biosynthetic pathways and rate of microbial growth (Sarwat, Ahmed, Aman, & Qader, 2013). Dextrans have various uses in the food, pharmaceutical and oil drilling industries (Aman, Siddiqui, & Qader, 2012). This wide spectrum of applications is due to physicochemical differences such as their molecular mass, which influences the viscosity and rheological properties of the polymer solution. Dextrans of high molecular mass are used for petroleum recovery; those of average molecular masses are employed in the chemical industry; whereas low-molecular mass dextrans find applications in the pharmaceutical and photographic industries (Vettori, Blanco, Cortezi, de Lima, & Contiero, 2012). In cosmetics, they are used as moisturizers and thickeners (Vu, Chen, Crawford, & Ivanova, 2009), and in the food industry, they are added to bakery products and confectionery to improve softness or moisture retention, to prevent crystallisation, and to increase viscosity, rheology, texture and volume (Pérez-Ramos et al., 2015). These HoPS also play an important role in stabilisation of frozen foods and can be used as films, to preserve the surface of fish, meat, vegetables or cheese from oxidation and other chemical changes (Harutoshi, 2013). In the medical field, dextrans are broadly used as blood plasma replacers/expanders and as heparin substitutes, for anticoagulant therapy. They can also produce iron dextrans, which are used in solution for treatment of human and veterinary anaemic deficiency. In addition, dextran sulphate has also shown an antiviral effect against the human immunodeficiency virus (Piret et al., 2000), and we have recently demonstrated that dextrans synthesized by LAB have potential as antivirals and immunomodulatory agents in trout (Nácher-Vázquez et al., 2015). Thus, dextrans can be used in the future for the manufacture of functional food and feed.

In the present work, we have performed a physicochemical characterization, and analysed the rheological properties, of dextrans produced by several strains of *Leuconostoc* and *Lactobacillus* isolated from food. Two physiological aspects were then tested *in vitro* for (i) the ability of the purified dextrans to immunomodulate human macrophages and, (ii) the influence of the dextrans on the ability of the producing LAB to interact with human enterocytes.

2. Materials and methods

2.1. Bacterial strains and growth conditions

Five LAB strains were used in this work. Three of them were isolated in Algeria: CM9 and CM30 from camel milk and SM34 from sheep milk. They were isolated using agar plates containing the MSE medium (Mayeux, Sandine, & Elliker, 1962) supplemented with 10% sucrose and vancomycin (30 μg mL^{-1}) at 30 °C for 72 h. These bacteria were identified as *Leuconostoc mesenteroides* (accession number in GenBank: KY083048 (CM9), KY082929 (CM30) and KY083047 (SM34)) by sequencing their 16S rRNA coding genes at Secugen (Madrid, Spain). The other two LAB were *Lactobacillus sakei* MN1, (CECT 8329) and *Lc. mesenteroides* RTF10, both isolated from meat products (Chenoll, Macian, Elizaquivel, & Aznar, 2007). LAB were grown at 30 °C in MRS (De Man, Rogosa, & Sharpe, 1960) containing 2% glucose (MRSG) or in MRS supplemented with 2% sucrose (MRSS) instead of glucose. For long-term storage at −80 °C, MRSG supplemented with 20% (v/v) glycerol was used. For EPS production, LAB were grown in defined CDM medium (Sánchez et al., 2008) supplemented with 0.8% sucrose (CDMS).

2.2. Production, purification, quantification and characterization of EPS

For production, LAB were precultured at 30 °C in MRSS to A_{600} = 2.0. Then, the bacteria were sedimented by centrifugation (12,000 × *g*, 10 min, 4 °C), resuspended in the same volume of fresh MRSS, diluted 1:100 in fresh CDMS medium and, incubated at 30 °C until the end of the exponential phase of growth. For EPS isolation and purification, cells were removed by centrifugation (10,651 × *g*, 30 min, 4 °C). The EPS was precipitated by addition of cold absolute ethanol to the supernatant (v/v) and storage at 4 °C for 24 h. After centrifugation (10,651 × *g*, 60 min, 4 °C), the sediment was air dried, dissolved in distilled water and dialysed for 3 days against ultrapure water, using a 12–14 kDa cut-off membrane. Then, the EPS was frozen at −80 °C and lyophilised until dry. Fractionation by size exclusion chromatography (SEC) was performed as previously described (Notararigo et al., 2013), as the final step of purification.

The concentration of EPS at each step of purification was determined by by the phenol-sulphuric acid method, using a glucose calibration curve, (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956). The amount of EPS present in the cultures supernatants was determined after their precipitation with three volumes of absolute ethanol and two washes with 80% (v/v) ethanol. During purification and after chromatographic fractionation, the EPS concentrations were determined in aqueous solutions at 2 mg mL^{-1}. In parallel, tests for detection of potential contaminants (DNA, RNA and proteins) were carried out using specific fluorescent staining kits and the Qubit® 2.0 fluorometric detection methods (ThermoFhiser Scientific) in the same solutions. This technique allows the detection of more than 0.5 μg mL^{-1} of DNA, 20 ng mL^{-1} of RNA, and 1 μg mL^{-1} of proteins.

The chemical characterisation of EPS was performed by: (i) determination of monosaccharide composition and phosphate content, (ii) methylation analysis and (iii) infrared (IR) spectroscopy as previously described (Notararigo et al., 2013).

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