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Studies on production of fructo-oligosaccharides (FOS) by gamma radiation processing of microbial levan

N. Jalan[a], Lalit Varshney[b], Nilanjal Misra[b], Jhimli Paul[b], D. Mitra[b], D.D. Rairakhwada[a], Z. Bhathena[a], Virendra Kumar[b,*]

[a] Department of Microbiology, Bhavan's College, Andheri (W), Mumbai 400058, India
[b] Radiation Technology Development Division, Bhabha Atomic Research Centre, Trombay, Mumbai 400085, India

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ABSTRACT

Microbial levan, a natural polymer of fructose, was produced and purified by alcohol precipitation from culture supernatants of *Bacillus megaterium* type 1 grown in an optimized liquid sucrose medium. GPC analysis showed that the yield of the major fraction of levan having molecular weight ~5000 D increased with increase in sucrose concentration in the broth. Levan subjected to ^{60}Co-gamma radiation as well as acid hydrolysis was investigated by rheometry, UV–visible spectrophotometry and gel permeation chromatography (GPC) techniques. Unlike most of the polysaccharides, levan powder exhibited good radiation degradation stability up to 150 kGy. Gamma irradiation of 10% levan aqueous solution at 250 kGy yielded 63.0% fructo-oligosaccharide (FOS) with an average molecular weight of 1250 D. Acid hydrolysis of levan using 0.5 N HCl for 60 min treatment time gave rise to the desired FOS with lower yield (23.1%) as compared to that obtained in gamma radiolysis process.

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1. Introduction

Levan is a natural polymer and found in different plants and microbial products. Levan is a homopolymer comprising of sugar fructose sub-units that are linked primarily by β-2,6-glycoside bonds and some β-2,1-glycoside bonds in their branches (Fig. 1 inset). It is produced via a trans-glycosylation reaction that occurs in the presence of enzyme levansucrase (EC 2.4.1.10) (Han, 1990). Depending on its source, levan can be either of low molecular weight (plant levans) or of high molecular weight with branching (microbial levans). Microorganisms, such as *Bacillus subtilis* (Elisashvili, 1984), *Zymomonas mobilis*, *Bacillus polymyxa*, *Erwinia herbicola*, *Rahnella aquatilis* (Kim, Kim, Lee, Song, & Rhee, 2000), *Acetobacter xylinum* NCI 1005 (Tajima, Uenishi, Fujiwara, Erata, & Munekate, 1998), etc., are the primary producers of microbial levan.

Levan being a natural polysaccharide finds applications in a wide range of fields such as food, pharmaceutical and cosmetics production (Park et al., 2003). Levan can also be used as an emulsifier, formulation aid, stabilizing thickener, surface-finishing agent, encapsulating agent; a carrier for colours and flavors in the food industry (Jang et al., 2001). Microbial levan has been known for many years; however, it has not been commercially produced and utilized, mainly because of non-availability of large quantity of low-cost source.

Low molecular weight levan, commonly known as fructo-oligosaccharides (FOS), is a non-digestible food fibre. It belongs to a class having 5–9 fructose molecules with molecular weight in the range 720 D–1296 D (Szwengiel, Czarnecka, & Czarnecki, 2004). Although it does undergo a partial depolymerisation under the influence of the stomach juice, it is not digested by the pancreas secretion or small intestine juices (Yamamoto et al., 1999), and, therefore, can be treated as a component of prebiotic nature. In vitro investigations showed that bifidobacteria could utilize FOS as a source of carbon. However, this depends on the degree of polymerization; a molecular weight of approximately 4500 D has been suggested as an upper limit for this purpose (Marx, Winkler, & Hartmeier, 2000). Fructo-oligosaccharide is also characterized by half the sweetness of saccharose. Therefore, it can find application as a sweetener for diabetic patients (Cha, Park, Yang, & Lee, 2001).

Presently, a wide range of polysaccharides are available in the market, which are widely used in the food, agriculture and other industries. Nevertheless, attempts are being made to find polymers, which apart from stabilizing, thickening and other properties, can also fulfill other functional aspects in food production. Microbial FOS, with its favourable prebiotic properties and structure that can qualify them as components of food cellulose, can be used as a superior alternative to the commercially available variants of levan.

* Corresponding author. Tel.: +91 022 25595689; fax: +91 022 25505338.
E-mail addresses: vkumar@barc.gov.in, vkrawat75@gmail.com, vkrawat@yahoo.com (V. Kumar).

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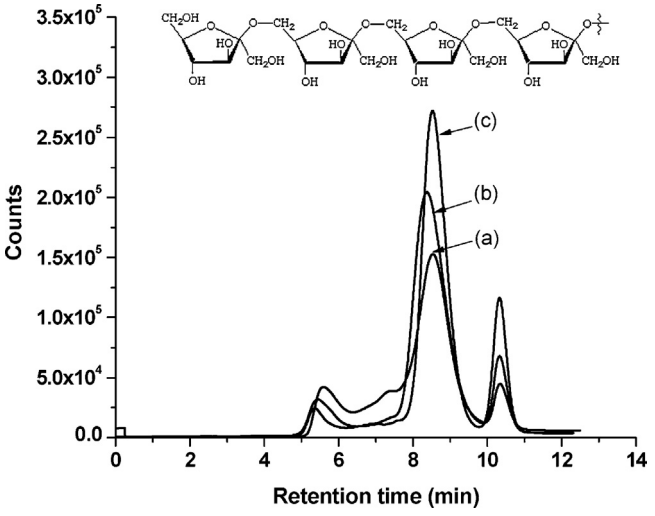

Fig. 1. GPC plots of levan samples produced from different concentrations of sucrose: (a) 10% sucrose, (b) 20% sucrose, (c) 30% sucrose. (Inset: general chemical structure of levan.)

It is well known that ionizing radiation treatment, including gamma irradiation, leads to degradation/depolymerization of polysaccharides, such as starch, cellulose, and pectin by cleavage of the glycosidic bonds and produces lower molecular weight fragments (Ananthaswamy, Vakil, & Sreenvasan, 1970; Charlesby, 1981; Cho, Kim, & Rhim, 2003; Sokhey and Hanna, 1993). The basic advantages of radiation induced degradation of polymers include better process control and elimination of the use of chemical reagents and the need for special equipments/setup for controlling the temperature, environment and additives (Charlesby, 1981). In the present work, to the best of our knowledge, we report herein for the first time, results of studies on gamma irradiation of microbial levan in solid as well as in aqueous solution phase and the feasibility of producing FOS of desired molecular weight. The results have been compared with acid hydrolysis of levan for production of FOS. Radiation processing of microbial levan to produce FOS has potential application in the field of food as sweetener.

2. Materials and methods

Levan was produced by cultivating a previously isolated strain of *Bacillus megaterium* type 1 in a high sucrose (10–30% sucrose w/v) liquid medium and purified as described by Yoshida, Suzuki, and Yagi (1990) and Jang et al. (2006). Briefly, the culture *Bacillus megaterium* type 1 was inoculated into optimized Han's medium containing known concentration of sucrose, 0.2% peptone, 0.2% yeast extract, 0.2% $(NH_4)SO_4$, 0.2% K_2HPO_4, 0.03% $MgSO_4$ and incubated at 30 °C under agitated and aerated conditions. Levan was harvested by centrifugation of the metabolic filtrate at 10,000 × g for 30 min. It was chilled and three volumes of cold ethanol were added. The mixture was homogenized by vortexing for 1 min and then centrifuged at 8000 × g for 10 min at 4 °C. Precipitated material was collected and re-suspended in 10 mL of distilled water and mixed again with three volumes of cold ethanol, for further purification. The microbial levan sample was subjected to multiple re-precipitation treatment in order to ensure maximum purity of the final levan sample. The purity of the microbial levan was tested by TLC method using butanol:ethanol:water (5:5:3) as mobile phase and fructose as a reference standard (Smith, 1958). The TLC result confirms the absence of other compounds in the levan samples. The polysaccharide precipitate was dried at 50 °C to a constant weight.

3. Experimental

3.1. Gamma irradiation

Samples of solid powder levan were taken in stoppered glass vials and then exposed to varying gamma radiation doses at a dose rate of 2.47 kGy h^{-1} using ^{60}Co Gamma Chamber-5000 (BRIT, India). Similarly, 10% aqueous solutions of levan were also irradiated at varying gamma radiation doses at a dose rate of 2.47 kGy h^{-1}.

3.2. Acid hydrolysis

For comparative study, levan sample was also subjected to acid hydrolysis treatment to get lower molecular weight fractions using 0.5 N and 1.0 N HCl at 25 °C. The course of levan hydrolysis with time was monitored using gel permeation chromatography (GPC).

3.3. Viscosity measurement

Viscosities of 10% solutions prepared from gamma irradiated solid levan and un-irradiated levan were determined using Brookfield DVII+ Pro viscometer with an ultra low adapter at 25 ± 1 °C and 85 rpm. For comparison, viscosities of gamma irradiated 10% levan solutions were also measured. Viscosity of distilled water was used as a reference.

3.4. UV–visible spectroscopy

The UV–visible spectra of irradiated and un-irradiated aqueous levan solutions (10%, w/v) were recorded between 200 nm and 500 nm using a double beam UV–visible spectrophotometer (Evolution 300, Thermoelectron, UK) against distilled water as a reference.

3.5. FTIR analysis

The IR spectra of control and gamma irradiated levan samples were recorded on diamond single reflectance ATR assembly in IR Affinity-1 spectrometer (Shimadzu) using resolution of 4 cm^{-1} and with data acquisition run of 25 scans for each sample.

3.6. GPC analysis: estimation of molecular weight of levan

Molecular weight and molecular weight distribution of levan samples were estimated by gel permeation chromatography (GPC) using PL aquagel–OH column (7.5 mm × 300 mm) and refractive index detector (Elite LaChrom, UK, Model L-2490). HPLC grade water was used as mobile phase at a flow rate of 1 mL/min. Solutions of un-irradiated and irradiated levan samples (1%, w/v) were filtered using Sartorius-Minisart filter unit (0.45 μm) and injected to the column through 20 μL loop. Calibration plot was established using standard PEG samples with known molecular weights. Evaluation of the chromatograms was carried out using size exclusion chromatography software (EZ Chrom Elite Software from Scientific Software, Inc.) to determine the molecular weights of the levan samples.

3.7. Particle size analysis

The hydrodynamic diameter of the irradiated and un-irradiated levan samples (2% aqueous solution at 25 °C) was determined using DLS measurements performed on a Malvern 4800 Autosizer employing 7132 digital correlator. The light source was He–Ne laser operated at 632.8 nm with an output power of 15 mW.

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