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β-D-glucan as an enteric delivery vehicle for probiotics

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ABSTRACT

Three strains of probiotics *L. casei*, *L. acidophilus* and *B. bifidum* were encapsulated in β-D-glucan matrix using freeze drying technique in order to increase survivability during their journey through GI tract. The encapsulation efficiency (%) of β-D-glucan varied significantly with Bifidobacteria showing low survival rate than Lactobacilli. SEM images revealed a partially collapsed structure with bacterial cells distributed randomly in the matrix. Further absorption band between 1300 and 900 cm^{-1} in FTIR spectra shows the presence of bacterial proteins and nucleic acids. For encapsulated β-D-glucan maximum degree of swelling was observed at pH 3 and 4, whereas when the pH value was increased to 6.5 the encapsulated β-D-glucan started their disintegration. In vitro release studies revealed that the microcapsules start to dissolve upon entry to the small intestine where it is needed most.

1. Introduction

Probiotics are defined as the microorganisms which, when consumed in sufficient amounts, confer health beneficial properties on the host [1]. Diets containing probiotics has raised the interest of consumers and there has been extensive research related to the subject from the past few years [2]. However, the challenging task for the researchers is that probiotics once consumed should reach in sufficient number to the target site within the host in order to exert beneficial effects. In this context encapsulation of probiotics within a wall material has proven to be of great importance and the efficacy of the process depends upon the type of wall material used and method of drying of cell suspension [3]. The selection of a wall material for encapsulation is always a challenge. It should have ability to retain the probiotics, maintain its structural integrity and resist the adverse environmental conditions of gastrointestinal tract [4]. The B-D-glucan is a very attractive material that can be used efficiently for the encapsulation of probiotics. Its uniqueness lies in its macroporous honey comb like structure that may trap the microbes much efficiently than any other wall materials [5]. B-D-glucans has ability to form controlled release systems as it is fermented by the microflora of colon [6,7]. Also β-D-glucans have low oral bioavailability and are thermo-reversible. Use of β-D-glucan gels has been widely studied for encapsulating various molecules like anthocyanin [8], protein [9], and antigen for drug delivery [10]. In our previous work we reported that β-D-glucan efficiently encapsulated Lactobacilli species in its matrix using emulsion technique [11]. Also in vitro studies have revealed β-D-glucan as a potential prebiotic, selectively promoting the growth of beneficial microflora in intestines in particular Lactobacilli and Bifidobacteria. Lactobacilli and Bifidobacteria are the two groups of bacteria that are mostly associated with GI tract [12]. The application of β-D-glucan for probiotic encapsulation is a new concept and is expected to protect the viability of microorganisms during their transit in the GI tract, processing and storage. There exists little or no available knowledge on in vitro release of encapsulated probiotics using β-D-glucan as a wall material. Therefore, in the present work β-D-glucan was utilized as a wall material for encapsulation of three probiotics strains *Lactobacillus casei, Lactobacillus acidophilus* and *Bifidobacterium bifidum* using freeze drying technique and assesses its survival during in vitro digestion.

2. Materials and methods

2.1. Materials

Barley β-D-glucan was procured from Grain-Frac Consulting, Edmonton, Canada. Probiotic bacterial cultures *Lactobacillus casei* (NCDC 297), *Lactobacillus acidophilus* (NCDC 14), and *Bifidobacterium bifidum* (NCDC 231) obtained from National Dairy Research Institute (NDRI), Karnal, India. Chemicals used in the study were procured from Sigma-Aldrich.

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2.2. Sub-culturing of probiotic strains

The freeze dried probiotic strains were grown in sterile MRS broth (HiMedia Laboratories Pvt Ltd, Mumbai, India) in CO_2 incubator (New Brunswick, Galaxy 170R, Eppendroff) at 37 °C in 3% CO_2 with 98% relative humidity. Probiotic bacteria were harvested by centrifugation at 8000g for 10 min at 4 °C, washed twice in sterile 0.85% saline water, and then stored at −18 °C in 5% glycerol.

2.3. Encapsulation

Encapsulation technique was carried according to the method described by Rajam et al. [13] with certain modifications. β-D-glucan (3 g) was dispersed in100 mL of sterile distilled water and stirred vigorously for 10 min on magnetic stirrer. To this mixture 1 mL of each prepared cultures (~9.91 log CFU/g) were mixed separately and again stirred gently for 5 min. Then the probiotic bacterial suspension dispersed in β-D-glucan solution was freeze dried (Operon IPS-55, Republic of Korea). The freeze dried β-D-glucan powders were collected in vials and stored at −18 °C.

2.4. Encapsulation yield and swelling index

The amount of viable probiotic bacteria encapsulated in β-D-glucan matrix was determined by sonicating 1 g of encapsulated powder dispersed in 9 mL of phosphate buffer saline (PBS pH 7.2) for 10 s in an ice bath (Power 60 W, Jain scientific, India), followed by enumeration on MRS agar plates after 48 h at 37 °C. Encapsulation yield (EY) (%) which is the ratio between the number of viable cells in the encapsulated material to the amount of viable cells added initially and was calculated using the given equation:

$$EY = N/N_o \times 100$$

Where N is the number of viable cells (log CFU/g) encapsulated, and N_o is the amount of viable cells (log CFU/g) added initially in the process.

Swelling index was determined by introducing 0.2 g (M_1) of freeze-dried encapsulated β-D-glucan in 100 mL of phosphate buffered saline at different pH values (3, 4, and 6.5) for a period of 2 h at 37 °C. After incubation the beads were withdrawn from the PBS with a spatula and weighed again (M_2). Swelling index (SI) is the gain in weight and was calculated by this formula:

$$SI = M_2 - M_1/M_1 \times 100$$

2.5. Attenuated total reflectance- Fourier transforms infrared (ATR-FTIR) spectroscopy

The spectra of encapsulated samples were recorded on an ATR-FTIR Spectrophotometer (CARY 630, Agilent Technologies, USA) at room temperature. The spectra were recorded within the range of 400–4000 cm^{-1} using Agilent Resolution-Pro MicroLab software (version B.05.2, Agilent Technologies).

2.6. Morphology study by scanning electron microscopy (SEM)

The samples were placed on an adhesive tape attached to a circular aluminum specimen stub. After coating vertically with gold- palladium, the samples were photographed at an accelerator potential of 5 kV using a scanning electron microscope (Hitachi S- 300H-Tokyo, Japan).

2.7. Thermotolerance of free and encapsulated bacteria

The heat resistance of encapsulated probiotics was determined as suggested by Sabikhi et al. [14] with certain modifications. One gram of freeze dried capsules and free cell suspension were transferred into test tubes containing 10 mL of sterile distilled water each and subjected to heat treatments at two different temperatures (60 and 70 °C) for 10 min. The viable count (log CFU/g) is determined by first sonicating the capsules in an ice bath for 10 s, followed by plating on MRS agar and enumerating after 48 h at 37 °C.

2.8. Survival of encapsulated probiotics during in vitro digestion

For in vitro cell release the simulated salivary juice (SSJ), gastric juice (SGJ), and intestinal juice (SIJ) is prepared following the method of Cheow et al. [15] with modifications. To mimic the human mouth conditions, 0.2 g of α-amylase is dissolved in 100 mL of phosphate buffered saline, pH 7.2 (8 gL^{-1} NaCl, 1.91 gL^{-1} $Na_2H PO_4^-$, 0.38 gL^{-1} $KH_2PO_4^-$). To 10 mL of this solution, 0.2 g of freeze dried sample was added and incubated at 37 °C for 5 min with constant shaking. Afterwards, the solution is carefully removed by pipetting and the pellet collected is dissolved in 10 mL of SGJ (simulated gastric juice). SGJ is prepared by dissolving 3 g/L pepsin in sterile NaCl solution (9 g/L) and adjusted the pH to 3.0 with 1.0 mol/L HCl. After an incubation period of 1 min and 60 min at 37 °C under constant agitation at 50 rpm, the cells released are determined. Again the gastric juice was removed and the pellet recovered was dissolved in 10 mL of SIJ (simulated intestinal juice) (3 gL^{-1} bile salts and 10 gL^{-1} pancreatin dissolved in PBS). The cells were incubated at 37 °C and an aliquot was taken after an interval of 25, 180, 240, & 300 min to mimic the conditions of intestines. After each step of digestion an aliquot of 1 mL was mixed with 9 mL of sodium chloride (0.85%). The cells released were determined by plating on MRS agar and expressed as log cfu/g.

2.9. Statistical analysis

Mean values, standard deviation, two-way analysis of variance (ANOVA) were computed using a commercial statistical package SPSS (IBM statistics 22). These data were then compared using Duncan's multiple range tests at 5% significance level.

3. Results and discussion

3.1. Encapsulation yield and swelling index

The encapsulation efficiency (%) of β-D-glucan for *L. casei, L. acidophilus* and *B. bifidum* were found to be 45.29, 34.41, and 25.36% respectively and varied significantly ($P < 0.05$) with Bifidobacteria showing low survival rate than Lactobacilli under similar drying conditions (Fig. 1). In our previous work we reported that emulsion technique using β-D-glucan as an encapsulating matrix proved helpful to obtain a good encapsulation yield [11]. Results revealed that using freeze drying technique for encapsulation of Lactobacilli and Bifidobacteria in β-D-glucan also ensured high cell survivability. During, freeze drying the water is directly converted to vapors by the process of sublimation thus causing least damage to encapsulated probiotic cells. The usage of β-D-glucan gels and cryogels as encapsulating matrices for various small and polymer molecules has been earlier reported due to its unique helical structure, thermostability, low oral bioavailability and ability to undergo fermentation by the colonic flora [8,9,16]. However its use to encapsulate the probiotics cells in particular Lactobacilli and Bifidobacteria using freeze drying technique for their targeted release seems to be interesting and has not been reported so far. Besides, it β-D-glucan also serves as a cryoprotector to protect the viability of