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Bacterial nanocellulose-pectin bionanocomposites as prebiotics against drying and gastrointestinal condition

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ABSTRACT

Various encapsulating materials have been suggested to protect probiotics, but the potential of nanomaterials is yet to be exploited. This study aimed to improve the survivability of *Bacillus coagulans* entrapping into bionanocomposites comprising of bacterial nanocellulose (BNC), pectin and *Schizophyllum commune* extract were investigated as new matrices to protect probiotics. The bionanocomposite design was optimized to obtain the highest prebiotic score and survivability of probiotic under drying process and gastrointestinal condition using the simplex-lattice mixture method. The optimal bionanocomposite formulation was obtained by mixing 20% pectin with 80% BNC. High survival rate of *B. coagulans* after microwave drying (99.43%) and sequential digestion under stimulated gastrointestinal fluids (94.76%) with optimum prebiotic score for *B. coagulans* (1.00) and for *Escherichia coli* (0.99), were obtained. Nanoscale properties of BNC, high crystallinity and available surface area resulted in high probiotic protection. Stability test during storage period at ambient temperature, 4 °C and −20 °C performed viability reduction, respectively, 1.3, 1.7 and 1.8 log CFU/g, which inferred the optimal bionanocomposite could be candidate as useful probiotics protection system in a variety of temperature during long time.

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1. Introduction

Gut flora might be an essential factor in pathological disorders of gastrointestinal tract. Nevertheless, some of them which are referred to as probiotics are useful in human health therapeutic intervention and prevention of some diseases [1]. During the production of probiotics, survivability is a major bottleneck since drying and passage through gastric juice have considerable effect on lowering the viability of probiotics [2]. The prebiotic known as non-viable dietary components which is targeted by the probiotic to be fermented, is fortified with certain gut flora [3]. The addition of protective compounds such as glucose and inulin to probiotic cultures can improve their viability during the manufacture and freeze drying process [2]. Encapsulation techniques such as emulsion, extrusion, spray drying and adhesion to support, are commonly applied to maintain probiotics viability through the gastrointestinal tract using various polysaccharides such as bacterial cellulose, gellan, xanthan, pectin, starch, alginate, carrageenan, and chitosan as the main wall materials. Structurally, interaction among the functional groups situated in the bacterial cell walls, coating materials and cross-linkers has a main role in establishing the modified probiotics [4]. However, selection of appropriate techniques and materials to minimize extra costs is necessary [5].

Pectin as a water soluble dietary fiber is naturally present in most plants, although citrus peel and apple pomace are the primary commercial sources of pectin [6]. Micro-encapsulated probiotics were effectively protected compared to the free cells. The coating of pectin microparticles with whey protein conferred protection on probiotics exposed on the gastrointestinal conditions [7]. Encapsulation of *Bifidobacterium bifidum* by mixing alginate, pectin and whey proteins, could protect the cells from freeze-drying and simulated gastric pH and bile salts on the gastrointestinal tract [8,9]. Lipid-protein-pectin layers improved thermal stability after spray drying and survivability during the gastrointestinal conditions by encapsulating *Lactobacillus salivarius* [10]. Under in vitro acidic conditions, pectin encapsulated *Lactobacillus plantarum* to efficiently improve the viability [11]. Probiotic bacteria entrapped in the gelatin of whey proteins typically achieved with polysaccharides such as alginate, pectin and carrageenan, were shielded

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from adverse conditions of the gastric and intestinal system [12]. Casein/pectin complex coated *Bifidobacterium lactis* and *Lactobacillus acidophilus* by spray drying, an efficient protection against the spray drying process and the simulated gastric juice [13]. As reported in recent studies, pectin is one of the encapsulating materials protecting probiotics; however, it can be combined with various prebiotics to create new composite materials with improved properties to protect probiotic under harsh conditions.

Bacterial cellulose (BC) with a high aspect ratio of high crystalline nanofibers, is a potent biomaterial for the synthesis of bionanocomposite encapsulating probiotics [14,15]; however, prebiotic property of BNC has not been fully studied yet. Nanocomposites which are biocompatible can be utilized to protect probiotics [16,17], but food science revolution has not yet led seriously to new bionanomaterials for applying nano-scale properties in enhancing the survivability of probiotics in unpleasant conditions.

Schizophyllum commune is an edible and medicinal mushroom, wildly growing from spring to autumn on dead wood, in coniferous and deciduous forest except Antarctica [18]. *S. commune* extract is widely sold as nutritional supplements and is beneficial for health because of anti-tumor mechanisms and anti-microbial substances [19,20]. *S. commune* extract based on the type of solvent can contain a mixture of polysaccharides such as glucan, phenols and proteins. Carbohydrate and protein contents of the extract are approximately 67% and 25%, respectively [18,21].

Unfortunately, this extract is not been investigated currently to encapsulate probiotic and measure prebiotic score.

Encapsulating materials containing soy protein and high methoxy pectin were optimized to delivery systems for probiotic bacterial [22]. Combination of the coating materials was optimized using the response surface methodology to maximize the thermotolerance of the probiotics under heat treatments [23]. Encapsulation of probiotic bacteria with a matrix optimized with Box–Behnken design increased their survivability by protecting them from harsh conditions [24]. In order to maintain the viability of probiotic, face centered composite design response surface methodology (FCCD-RSM) was employed to optimize the sodium alginate concentration and aloe vera gel composition for better encapsulation of *L. acidophilus* [25]. Today, optimizing the composition of various encapsulating materials is required for better probiotic protection. To the best of our knowledge, there are no reports on optimization of encapsulating materials containing bacterial nanocellulose (BNC).

In this study, in order to enhance probiotic survivability with prebiotic BNC based biomaterial, we used bacterial nanocellulose, pectin and *S. commune* aqueous extract to formulate prebiotic bionanocomposites that were designed by using simplex-lattice mixture method for entrapment of probiotic cells. Different bionanocomposites were designed and optimized to increase survivability of the dried probiotic as it enters into the gastrointestinal tract.

2. Materials and methods

2.1. Materials

Bacillus coagulans IBRC-M 10807 as a probiotic strain and *Escherichia coli* IBRC-M 10208 as an enteric strain were obtained from Iranian Biological Research Center (IBRC). Pectin was extracted from citrus peel and dried using previous established method [26]. Bacterial nanocellulose with fibril diameter average of 50 nm was purchased from Nano Novin Polymer Co. (Sari, Iran). *S. commune* extract was prepared by extraction of dried *S. commune* powder (5 g) in 50 mL distilled water at 40 °C for 1 h. Pepsin from porcine gastric mucosa (0.7 FIP-U/mg), and pancreatin from porcine pancreas (350 FIP-U/g protease, 6000 FIP-U/g lipase, 7500 FIP-U/g amylase) were purchased from Merck (Darmstadt, Germany). Bile salts and TSB acquired from Sigma–Aldrich (St. Louis, MO, USA) and Oxoid Ltd. (Basingstoke, Hampshire, UK), respectively.

2.2. Prebiotic score

Prebiotic score was defined according to the procedure established previously [27] with the following changes. In order to determine prebiotic score, the assay was performed by adding 1% (v/v) of an overnight culture of the bacterial strains to separate tubes containing 1 mL of TSB with 0.1% (w/v) glucose or 0.1% (w/v) prebiotic. The tubes were incubated at 37 °C under aerobic condition. After 24 h of incubation, samples were enumerated on TSA. Each assay was repeated three times. The prebiotic score was determined using the following equation [27]:

$$\text{P.S. score} = \frac{[(\text{bacteria}\log(\text{CFU mL}^{-1})\text{ on the prebiotic at 24 h}) - (\text{bacteria}\log(\text{CFU mL}^{-1})\text{ on the prebiotic at 0 h})]}{[(\text{bacteria}\log(\text{CFU mL}^{-1})\text{ on glucose at 24 h}) - (\text{bacteria}\log(\text{CFU mL}^{-1})\text{ on glucose at 0 h})]} \quad (1)$$

2.3. Preparation of free and entrapped cells

B. coagulans was grown overnight in TSB medium at 37 °C. The cells were centrifuged at 6000 rpm for 10 min, then washed with sterile saline solution (0.5%, w/v), and resuspended in the same solution.

In order to entrap the probiotic cells into the bionanocomposite matrix, the designed composition was transferred to a 2.0 mL Eppendorf tube and autoclaved, then added to the same volume of the bacterial suspension and mixed well using vortex. The free and entrapped cells were dried for 7 min at 400 W microwave power using microwave oven (NN-ST342, Panasonic Co., Japan) [28].

2.4. Preparation of simulated gastric and intestinal juice

Gastrointestinal conditions were simulated as described previously [29]. In brief, simulated gastric juice was prepared by suspending pepsin in sterile saline (0.5%, w/v) to a final concentration of 3 g L^{-1} and pH was adjusted to 2.00 using 1 N HCl. Simulated intestinal juice was prepared by suspending pancreatin USP in sterile saline to a final concentration of 1 g L^{-1} and adding 4.5% bile salts, then the pH was adjusted to 8.00 using sterile 1 N NaOH.

2.5. Survivability of free and entrapped cells after drying

For the hydration of the samples, they were immersed in 1 mL of sterilized peptone water (0.1%, w/v) for 2 h. The sample was serially diluted with the peptone water for cell counts. The diluted sample was transferred onto TSA plate and incubated at 37 °C for 48 h. Individual colonies appeared were counted as colony forming units per gram of the sample (CFU/g) and the results were reported as Log_{10} values.

The survival rate was calculated according the following equation [30]:

$$\text{Survival rate} = \frac{[\text{bacteria }\log(\text{CFU/g})\text{ after drying}]}{[\text{bacteria }\log(\text{CFU/g})\text{ before drying}]} \times 100 \quad (2)$$

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