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Improved Viability and Thermal Stability of the Probiotics Encapsulated in a Novel Electrospun Fiber Mat

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ABSTRACT: For the enhancement of the probiotics' survivability, a nanostructured fiber mat was developed by electrospinning. The probiotic *Lactobacillus plantarum* was encapsulated in the nanofibers with fructooligosaccharides (FOS) as the cell material. Fluorescence microscope image and scanning electron microscopy (SEM) showed that viable cells were successfully encapsulated in nanofibers (mean diameter = 410 ± 150 nm), and the applied voltage had no significant influence on their viability ($P > 0.05$). A significantly improved viability (1.1 log) was achieved by incorporating 2.5% (w/w) of FOS as the electrospinning material ($P < 0.001$). Additionally, compared with free cells, the survivability of cells encapsulated in electrospun FOS/PVA/*L. plantarum* nanofibers was significantly enhanced under moist heat treatment (60 and 70 °C). This study shows that the obtained nanofiber is a feasible entrapment structure to improve the viability and thermal stability of encapsulated probiotic cells and provides an alternative approach for the development of functional food.

KEYWORDS: *oligosaccharide, electrospinning, probiotic, encapsulation, viability, stability*

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1. INTRODUCTION

Probiotics are defined as live microorganisms which, when administered in adequate amounts, can possess health benefits by keeping the balance of microorganisms in human gastrointestinal tract, preventing some pathologies, and boosting the host immune system.[1−3] Good probiotic viability and activity are considered essential for their optimal functionality. The viability of probiotics is, however, significantly affected due to harsh environmental conditions such as those involved in food processing and storage conditions.[4−6]

In recent years, microencapsulation has been demonstrated to be a promising way for bacterial cell protection.[7] Several research reports have reported probiotic microencapsulation by different techniques, such as extrusion, emulsification, and spray drying.[8−10] However, many of these microencapsulation methods involve the use of high temperatures or organic agents, which can result in significant mortality of probiotic cells.[11−13] Electrospinning, a mild and cost-effective technique, has already been utilized as an alternative encapsulation method for sensitive food and bioactive compounds in the past few years due to its several advantages.[14−17] Additionally, electrospinning has recently emerged as a potential way for encapsulating bacterial cells.[18,19] However, it is well-known that a sufficient number of viable probiotic cells is the key factor for exhibiting full functionality and health effects. Prebiotics are nondigestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of probiotics.[20] Several studies have proven that the addition of prebiotic provided improved protection for the active probiotic organisms.[21,22] While encapsulating probiotics in electrospun fibers has been approved to be feasible, no systematic report has been conducted with an aim of further improving the viability of the encapsulated probiotics by incorporating a prebiotic as an electrospinning material. Additionally, temperature is one of the key factors which affect the viability of probiotics. The favorable temperature for the growth of most probiotics is in the range of 37−43 °C.[23] However, some thermal processing (above 45 °C) in the food industry will result in a major loss of cell viability, thus compromising its functionality. Therefore, the improvement of thermal stability of encapsulated probiotics is also very important for its application.

Herein, we first investigated the prebiotic activity of three commercial oligosaccharides (FOS, GOS, and IMO) to obtain a desired increase in *Lactobacillus* and *Bifidobacterium* populations, and then, the suitable oligosaccharide was chosen as the electrospinning material. Then, the electrospinnability of the selected prebiotic and its effect on the properties of electrospinning solutions were studied. Finally, we selected *L. plantarum* as a model probiotic and encapsulated them in electrospun fibers under sterile conditions. Since electrospinning requires an electrostatic voltage, the influence of different voltages on the viability of probiotic was also investigated. The obtained fibers were characterized by scanning electron microscopy (SEM), thermogravimetric analysis (TGA), attenuated total reflection-Fourier transform infrared spectroscopy (ATR-FTIR), and

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inverted fluorescence microscopy. The effect of fructooligosaccharides (FOS) on the viability of encapsulated *L. plantarum* cells was explored. Furthermore, the survivability of the encapsulated cells in electrospun FOS/PVA/*L. plantarum* nanofibers under moist heat treatment was investigated.

2. MATERIALS AND METHODS

Poly(vinyl alcohol) (PVA) (hydrolysis was 98−98.8%) was obtained from Sinopharm Chemical Reagent Co., Ltd. (Beijing, China). FOS (90.1%), galactooligosaccharides (GOS) (95.8%), and isomaltooligosaccharides (IMO) (80.3%) were purchased from New Francisco Biotechnology Co., Ltd. (Yunfu, China). Four *Lactobacillus* strains (*L. casei*, *L. paracasei*, *L. plantarum*, and *L. acidophilus*) and two *Bifidobacterium* strains (*B. adolescentis* and *B. bifidum*) were kindly donated by Prof. Jiguo Yang's laboratory of the School of Food Science and Engineering, South China University of Technology.

2.1. Screening of Prebiotic. Oligosaccharide (2% w/v) was dissolved in the de Man−Rogosa−Sharpe (MRS) medium that lacked glucose, followed by inoculation of 1% (v/v) of overnight cultures of *Lactobacilli* strains or *Bifidobacteria* strains. Subsequently, the probiotics were cultured in anaerobic atmosphere at 37 °C for 24 h, and then cell growth was measured by assessing the optical density (OD) of the cultures at 600 nm (OD_{600}) and their pH values.

The growth of probiotic on different oligosaccharides was compared to that on glucose using the growth index (GI) (eq 1) to verify the prebiotic effect of different oligosaccharide:[24]

$$GI = \frac{(A_{P24} - A_{P0}) - (A_{C24} - A_{C0})}{(A_{G24} - A_{G0}) - (A_{C24} - A_{C0})} \quad (1)$$

where A_{P24}, A_{G24}, and A_{C24} represent the absorbance of an appropriate medium either containing the sample prebiotic (P) or glucose (G), or lacking any carbohydrate (control, C) after incubation for 24 h, respectively. A_{P0}, A_{G0}, and A_{C0} stand for the absorbance readings at 0 h.

2.2. Preparation and Characterization of Electrospinning Solutions. A 6% PVA solution was obtained by dissolving PVA in sterile deionized water under constant stirring using a magnetic stirrer (RT5, IKA, German) at 60 °C for 30 min and then at 80 °C for 1 h as described by Feng et al.[15] Then, 0.5−3% (w/w) FOS was dissolved in the PVA solution by constant stirring at room temperature, and then the solution was autoclaved to achieve sterility. For the FOS/PVA/*L. plantarum* electrospinning solution, 1 mL of the culture medium (10^9 CFU/mL) was added into 20 mL of the obtained 2.5% FOS/6% PVA solution or 6% PVA solution, with stirring for 10 min under sterile conditions. The viscosities and conductivities of PVA and FOS/PVA solutions were measured by a Brookfield digital viscometer (model DV-II+Pro, USA) and a conductivity meter (DDS-11A, China), respectively. For viscosity measurements, the temperature (25 ± 0.1 °C) of the solution in the container was controlled by a water bath (EYELA, Tokyo Rikakikai Co. Ltd., Japan) and the solution was stirred at 140 rpm with a S21 spindle.

2.3. Electrospinning. Electrospinning was performed by using a voltage power supply (ES50P-5W/DAM, Gamma, USA), and the solution was loaded in a plastic syringe (10 mL) with a 20 gauge steel needle. The feed rate of the solution was varied from 0.3 to 0.6 mL/h, which was controlled by a syringe pump (NE-300, New Era Pump Systems Inc., USA). Other electrospinning conditions were a voltage of 16 kV and a distance between the needle tip and collector of 14 cm. The obtained fibers were collected with a grounded plate covered by aluminum foil. The electrospinning process was carried out at 25 ± 1 °C, and the relative humidity was around 40−50%. The obtained film was stored in a sterile box.

2.4. Characterization and Measurement. The morphologies and fiber diameters of the electrospun nanofibers were characterized using an SEM instrument (S-3700N, Hitachi, Japan). First, electrospun nanofibrous film was coated with Pt for 40 s using a sputter coater (K550, Emitech, U.K.) under vacuum. Then, the morphology was examined at an accelerating voltage of 15 kV, and Nano Measure 1.2 software was utilized to measure the fiber diameter. The diameter distribution of the fibers was then calculated by analyzing around 50 fibers from the SEM images.

The interactions between components were analyzed by a Bruker Model Equinox 55 FTIR spectrophotometer (Bruker Co., Ettlingen, Germany). With regard to the electrospun nanofilm, ATR (attenuated total reflection) was utilized, while for the PVA, FOS, and *L. plantarum*, a KBr disk was adopted. FTIR (Fourier transform infrared spectroscopy) was performed in the middle infrared region with a wavenumber range of 4000−500 cm^{-1} and spectral resolution of 4 cm^{-1}.

The thermal behavior of different samples was determined by thermal gravimetric analysis (TGA-Q5000, TA Instruments, USA). Samples were heated from 25 to 700 °C at a heating rate of 20 °C/min under a nitrogen atmosphere. The obtained data were processed by TA Universal Analysis software.

The presence and distribution of bacterial cells stained with Rhodamin 123 were observed using an IX73 inverted fluorescence microscope (Olympus Corp., Tokyo, Japan) under epifluorescent illumination to check whether *L. plantarum* cells were properly encapsulated within the fibers.

2.5. Viability Study. Electrospun FOS/PVA film (200 mg) with different concentrations of FOS (0−3%, w/w) was added in the fresh MRS medium that lacked glucose, and then, 1% (v/v) of precultured microorganism with a concentration of approximately 10^8 CFU/mL was added. The blank control was constructed by adding no films into the tubes. After that, the tubes were incubated in anaerobic atmosphere at 37 °C for 24 h. OD_{600} and pH values of the culture medium were measured.

The effect of FOS both in electrospun nanofilm and electrospinning solution on the viability of the encapsulated *L. plantarum* was investigated. As for electrospun FOS/PVA/*L. plantarum* nanofilm, 50 mg of the film was added into the MRS medium that lacked glucose; after that, all of the tubes were incubated in an anaerobic atmosphere at 37 °C for 24 h. Then, the OD_{600} values of the cultures were measured, and the cultures were diluted, followed by them being spread on the MRS agar plate and incubated in an anaerobic atmosphere at 37 °C for 24 h. Similarly, 500 μL of FOS/PVA/ *L. plantarum* solution was utilized during the operation mentioned above. Additionally, 20 mg of the electrospun FOS/PVA/*L. plantarum* nanofilm obtained by utilizing different voltages (10−16 kV) was added into the MRS medium, followed by incubation in an anaerobic atmosphere at 37 °C for 24 h, and then the OD_{600} of different cultures was measured.

2.6. Survival of Free and Encapsulated Cells under Moist Heat Treatment. Free cells and encapsulated cells in FOS/PVA/*L. plantarum* nanofilm were added into the unsealed tubes. Then, the tubes were incubated in a water bath (45, 60, and 70 °C) for 30 min to evaluate the moist heat resistance of the samples. After heat treatment, the samples were transferred into the tubes containing 5 mL of MRS broth each, followed by incubating them in an anaerobic atmosphere at 37 °C for 24 h, and then, the count of viable cells was performed as previously described in section 2.5.

2.7. Statistical Analysis. Experiments were performed in triplicate, and the obtained data were presented as the mean ± standard deviation. Statistical analysis was performed with SPSS Statistics Software (version 16.0). One-way ANOVA was used to determine the difference between groups. Differences were considered significant if $P < 0.05$.

3. RESULTS AND DISCUSSION

3.1. Screen of Effective Prebiotic. At first, the oligosaccharide with the highest growth index value among the three commercial oligosaccharides was supposed to have the best prebiotic effect on the tested probiotics and, thus, was selected for subsequent studies. As shown in Figure 1, the growth of probiotics varied considerably depending on the oligosaccharide substrate. An increase in OD_{600} of the culture broth and a decrease in pH of the culture media, as a result of short chain fatty acid production, reflected the ability of the probiotic to

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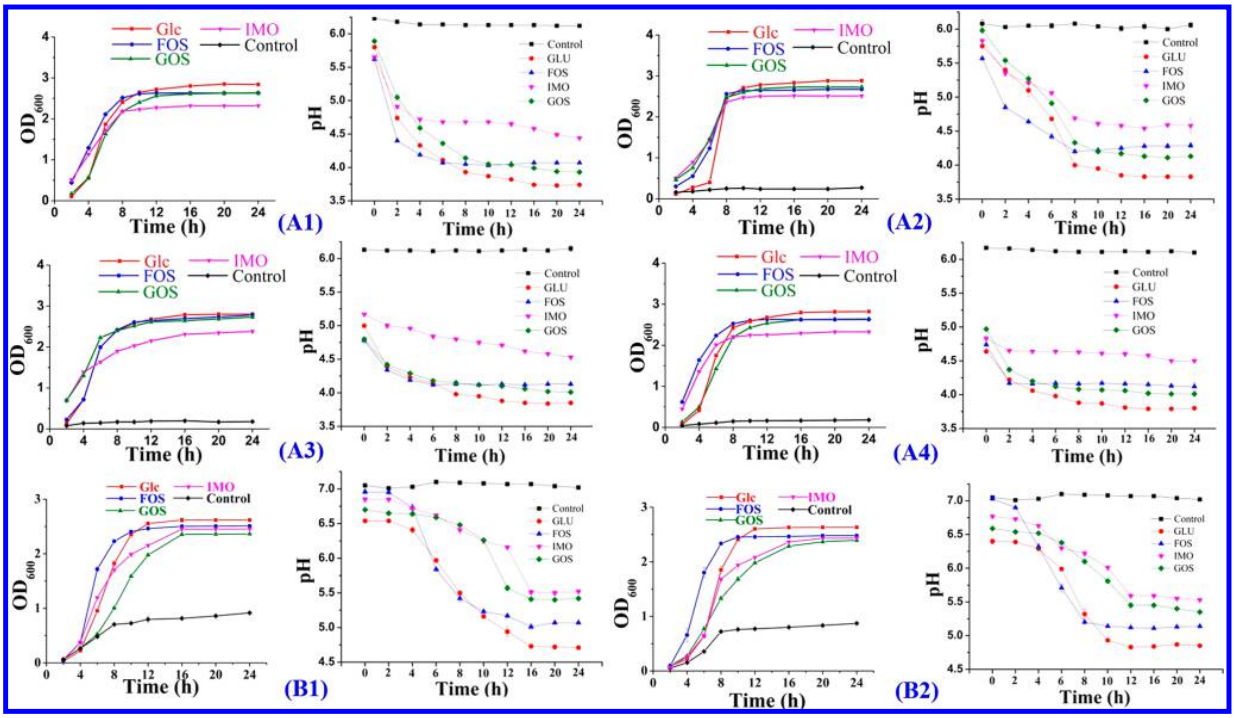

Figure 1. Effect of three different oligosaccharides on the population growth of *L. casei* (A1), *L. paracasei* (A2), *L. plantarum* (A3), *L. acidophilus* (A4), *B. adolescence* (B1), and *B. bifidum* (B2) and pH of the culture medium. (Glc, glucose; FOS, fructooligosaccharide; GOS, galactooligosaccharide; IMO, isomaltooligosaccharide; Control, no glucose or prebiotic).

Table 1. Growth Promotion of Different Bacteria Strains by Three Oligosaccharides[a]

	growth index					
oligosaccharides	*L. casei*	*L. paracasei*	*L. plantarum*	*L. acidophilus*	*B. adolescence*	*B. bifidum*
FOS	93.54 ± 0.21 c	92.04 ± 0.52 b	99.01 ± 0.27 c	92.68 ± 0.24 b	93.39 ± 0.21 c	91.81 ± 0.16 c
GOS	91.72 ± 0.13 b	91.35 ± 0.49 b	90.95 ± 0.16 b	92.61 ± 0.50 b	84.79 ± 0.33 a	86.54 ± 0.45 a
IMO	79.90 ± 0.16 a	78.92 ± 0.22 a	80.40 ± 0.16 a	80.58 ± 0.88 a	90.06 ± 0.33 b	88.77 ± 0.40 b

[a]Mean ± standard deviation with different lowercase letters that indicate significant difference ($P < 0.05$) between the parameter tested.

grow on the substrates.[25] In addition, the growth indices of the tested *Lactobacillus* and *Bifidobacterium* strains were of the highest values when grown on FOS compared to those grown on other oligosaccharides (Table 1). In regard to the promotion effect of FOS, OD_{600} increased gradually with the increase of FOS concentration, while there was no obvious change with further increases of FOS concentration (Figure 2). Hence, FOS was selected as an efficient prebiotic for further experiments, and it is important to choose the suitable concentration of FOS for producing electrospun fibers with proper prebiotic effect.

3.2. Fabrication and Morphology of Electrospun Film. The aim of this study was to encapsulate the probiotic by using the screened oligosaccharide as the electrospinning material. Viscosity and conductivity are two key factors that influence the electrospinning. The balance between them are crucial for obtaining electrospun nanofibers with good morphology.[26] However, our preliminary study showed that the low viscosity and low conductivity of FOS make it difficult to electrospin. A high molecular weight polymer, PVA, was added to improve the electrospinning process. The conductivity of the solution was decreased with the addition of FOS. However, there was no obvious change for the viscosity when the added FOS was less than 2.5% (Table 2). The obtained FOS/PVA nanofilm and its fiber distribution are shown in Figure 3. The growth promoting properties of the FOS/PVA films with different concentrations of FOS on two *Lactobacillus* strains (*L. casei* and *L. plantarum*) and two *Bifidobacterium* strains (*B. adolescence* and *B. bifidum*) were investigated to verify whether the electrospun FOS/PVA fibers retained prebiotic activity. Figure 4 reveals that there is no significant difference between the PVA film and the control group. That is to say, PVA film had no prebiotic or toxic effect on the probiotic strains tested. Similarly to the FOS, the prebiotic effect of FOS/PVA film was increased with the increase of FOS concentration in the FOS/PVA film, reaching a maximum at 2.5% FOS. Further increases in FOS concentration, above 2.5%, did not further enhance bacteria concentration. The result mentioned above also demonstrated that the electrospinning process had no significant influence on the prebiotic effect of FOS. Hence, 2.5% FOS was selected as the electrospinning material to encapsulate probiotics. The SEM micrograph and diameter distribution of the as-spun nanofiber (2.5% FOS/6% PVA/*L. plantarum*) are shown in Figure 5. Compared to the electrospun 2.5% FOS/6% PVA fibers (Figure 3A), the incorporation of *L. plantarum* into the electrospinning solutions led to beaded fiber morphology

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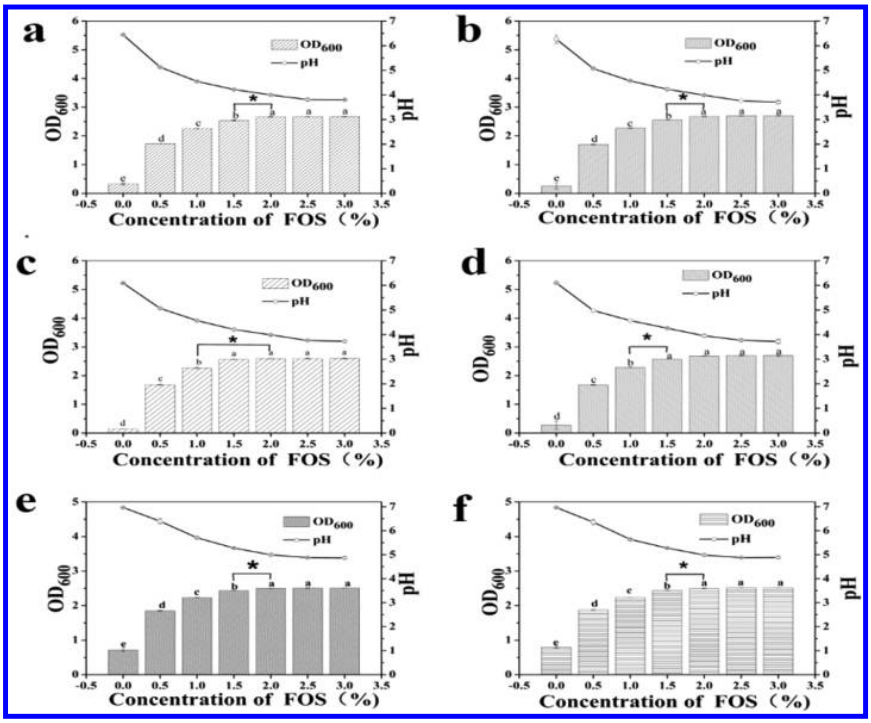

Figure 2. Prebiotic effects of different concentrations of FOS on different probiotics ((a) *L. casei*; (b) *L. paracasei*; (c) *L. acidophilus*; (d) *L. plantarum*; (e) *B. adolescence*; (f) *B. bifidum*). Different lowercase letters above the column indicate significant difference ($P < 0.05$).

Table 2. Effect of FOS Concentration on the Properties of PVA/FOS Solutions[a]

% PVA (w/v)	% FOS (w/v)	viscosity (cP)	conductivity (μS/cm)
6		129.4 ± 1.6 a	594.5 ± 12.0 d
6	0.5	130.3 ± 0.6 a,b	573.0 ± 9.9 b,c,d
6	1.0	130.2 ± 0.2 a,b	566.5 ± 6.4 b,c
6	1.5	130.4 ± 0.1 a,b	556.0 ± 7.1 a,b,c
6	2.0	131.2 ± 0.1 b	553.0 ± 8.5 a,b,c
6	2.5	131.1 ± 0.4 a,b	548.0 ± 11.3 a,b
6	3.0	131.2 ± 0.3 b	542.0 ± 8.5 a

[a]Mean ± standard deviation with different lowercase letters that indicate significant difference ($P < 0.05$) between the parameter tested.

and thus resulted in a local widening of the nanofibers. A similar phenomenon had been previously demonstrated.[18] The encapsulation of probiotic cells protected them from destruction by the harsh conditions. Meanwhile, the fluorescence microscope image of the encapsulated *L. plantarum* in the electrospun fibers demonstrated that the living *L. plantarum* cells that emitted yellow-green fluorescence were encapsulated in electrospun fibers, indicating that electrospinning is a promising approach for encapsulating probiotics.

3.3. Characterization of the Electrospun Film. ATR-FTIR was employed to study the peak shifts that could reveal the interactions between FOS, PVA, *L. plantarum*, and the nanofilms, such as hydrogen bonding. As can be seen in the spectrum of PVA in Figure 6a, the characteristic peak at 3463 cm^{-1} indicated that the O−H stretching formed between PVA and water. Similarly, FOS and *L. plantarum* also had the characteristic absorption peak at 3426 and 3414 cm^{-1}. However, a red shift was observed for the O−H stretching peak in the spectrum of FOS/PVA nanofilm (3317 cm^{-1}) and FOS/PVA/*L. plantarum* nanofilm (3319 cm^{-1}) compared with the spectrum of PVA (3463 cm^{-1}), FOS (3425 cm^{-1}), and *L. plantarum* (3413 cm^{-1}), indicating that more hydrogen bonds were formed between PVA, FOS, and *L. plantarum*. Additionally, the spectrum of *L. plantarum* also displayed an absorption region between 900 and 1300 cm^{-1}, which corresponded to the bacterial proteins and nucleic acids.[27] Similarly, a typical band in the region of 900−1200 cm^{-1} in the spectrum of FOS corresponded to the C−C and C−O bonds.[28] These absorption peaks were also present in the spectrum of FOS/PVA film, which suggested the existence of FOS in the film. In addition, compared to the peaks between 900 and 1300 cm^{-1} of the FOS/PVA film, this region of the FOS/PVA/*L. plantarum* had a greater intensity, a narrower peak, and exhibited a blue shift, which was similar to reports of a previous study,[29] and elucidated that the cells were encapsulated in the fibers. This result was supported by the SEM image and fluorescence microscope image of the electrospun FOS/PVA/*L. plantarum* fibers (Figure 5). The thermal properties of different components and the obtained electrospun fibers were measured by TGA, and the weight loss and its derivative curves are presented in Figure 6b and c. As depicted in Figure 6b, the *L. plantarum* degraded in three steps, and these are evidenced by the distinct reaction peaks observed at around 51, 217, and 319 °C in the DTG curves (Figure 6c). Additionally, as for the electrospun FOS/PVA/*L. plantarum*, the degradation temperature was significantly increased as noticed in Figure 6b and c ($P < 0.05$). This phenomenon can be attributed to the encapsulation of the *L. plantarum* cells within the electrospun fibers.

3.4. Effect of the Addition of FOS on the Viability of Encapsulated Probiotic Cells. Two approaches were utilized in this paper to protect the probiotics and enhance their viability. First, electrospinning, known as a mild and simple

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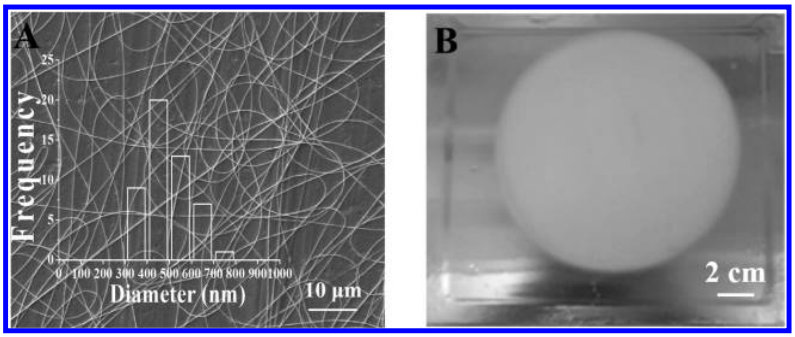

Figure 3. Images of the obtained electrospun 2.5%FOS/6%PVA film ((A) SEM image; (B) deposited on the aluminum foil).

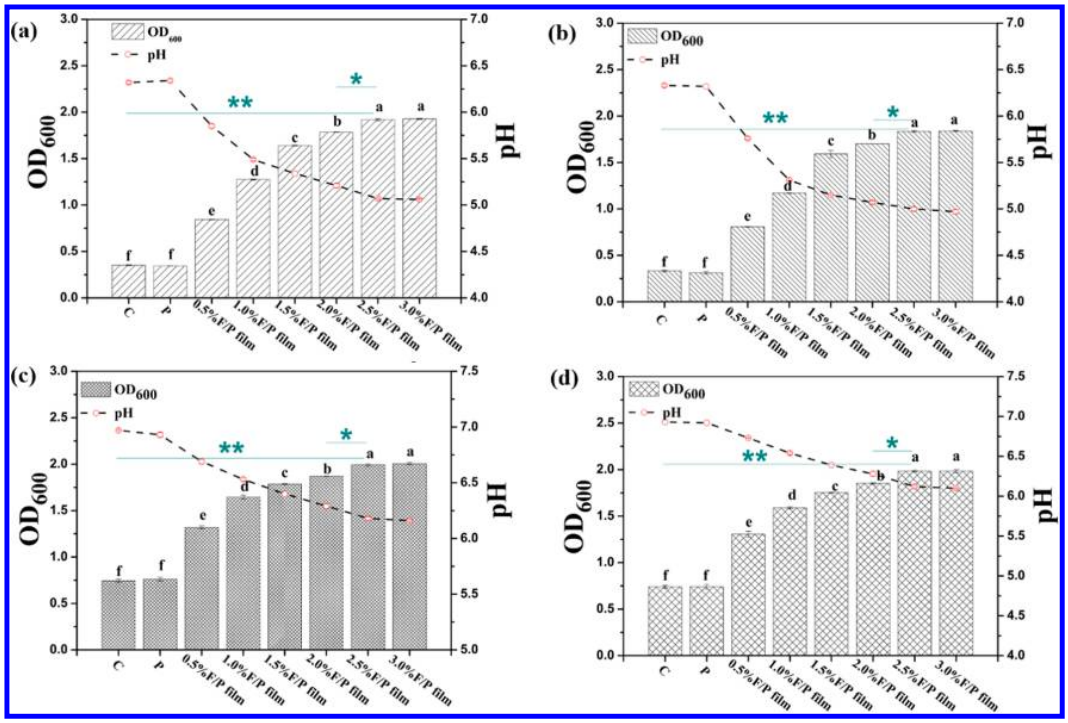

Figure 4. Prebiotic effects of the electrospun FOS/PVA film with different FOS concentrations on different *Lactobacillus* and *Bifidobacterial* strains ((a) *L. casei*; (b) *L. plantarum*; (c) *B. adolescence*; (d) *B. bifidum*). (C, control; P, PVA film; F, FOS; F/P film, FOS/PVA film; $*P < 0.05$; $**P < 0.001$). Different lowercase letters above the column indicate significant difference ($P < 0.05$).

technology, was applied to encapsulate the probiotics. However, the electrospinning process requires the use of high-voltage electric fields to produce electrically charged jets of solutions, and ultrathin fibers are obtained by the evaporation of solvent.[30] Hence, it was necessary to examine whether the applied voltage significantly influenced the viability of encapsulated probiotic cells. The results demonstrated that as the applied voltage increased from 10 to 16 kV, there was no significant difference for the viability of *L. plantarum* cells (Figure 7a). Moreover, the loaded cells retained high viability even after being subjected to a high voltage. Second, an efficient oligosaccharide with excellent prebiotic effects, FOS, was used here as an electrospinning material to encapsulate the probiotic cells. Furthermore, we evaluated the survivability of the encapsulated *L. plantarum* in electrospun fibers and the prebiotic effect of FOS on the viability of encapsulated cells. As depicted in Figure 7b, the number of surviving probiotic cells in the presence of FOS was significantly higher than those without FOS for both fibers and the electrospinning solutions ($P < 0.001$). The viability has improved 1.1 log for FOS/PVA/*L. plantarum* film over the film without FOS. Hence, the two strategies explored here provided a new development idea for the functional food industries, especially as probiotic supplements used in the food products with low water activity.

3.5. Survival of Free and Encapsulated Cells under Moist Heat Treatment. The recommended minimum number of live probiotic cells in food to confer health benefits to the host was 10^6–10^7 CFU/mL or grams of food.[31] However, cell survivability tends to be significantly influenced by some heat processing methods in the food industry, thus compromising its functionality. In regard to this, the encapsulation technique is an attractive way to enhance the thermal stability of cells. Therefore, the protection efficiency of probiotic cells by the electrospun FOS/PVA/*L. plantarum* nanofilm under the moist heat treatment was investigated. As shown in Table 3, there was no significant difference between the cell viability of

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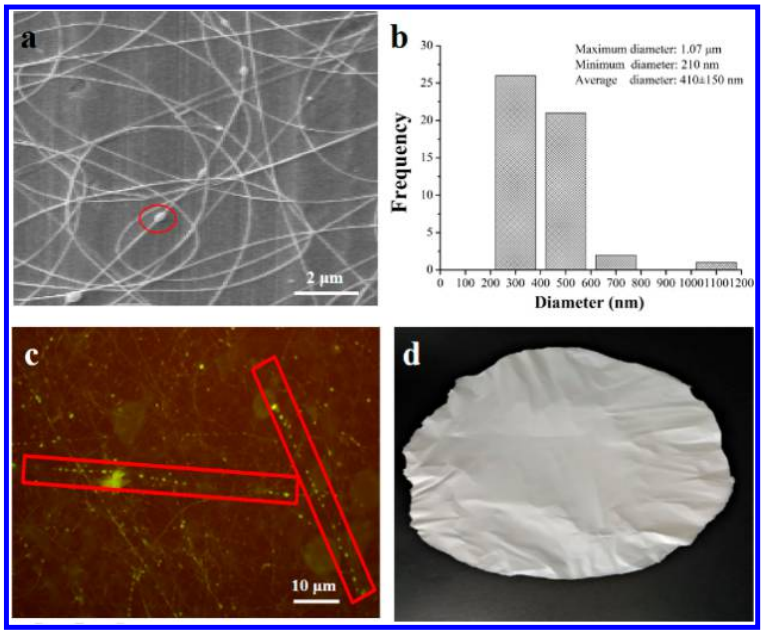

Figure 5. SEM image (a), fiber diameter distribution (b), and inverted fluorescence microscope image (c) of the FOS/PVA/*L. plantarum* nanofibrous film (d).

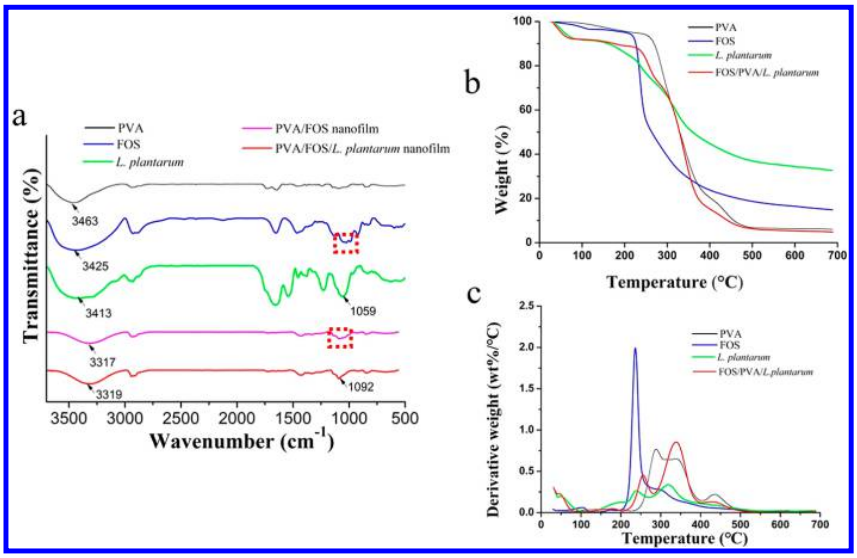

Figure 6. FITR spectra (a), TG (b), and DTG (c) curves of different samples.

free cells and encapsulated cells when the temperature was at 45 °C, while the free cells obviously suffered a significant loss of viability by exposure to temperatures above 45 °C ($P < 0.05$). However, the viability of *L. plantarum* cells encapsulated in the FOS/PVA/*L. plantarum* nanofilm, which exhibited a high capacity to protect the loaded cells, was reduced by only 0.04−2.71 log CFU/mL, and the probiotic count was also above 6 log CFU/mL when the temperature was at 70 °C. This might be due to the encapsulation of nanofiber, which supplied an barrier to inhibit the diffusion of hot moisture into the nanofibers, thus improving the thermal stability of probiotic cells during moist heat treatment. This result revealed that the obtained electrospun fibers was an alternative carrier for ensuring a good stability of the encapsulated cells under the moist heat treatment.

In summary, the above results demonstrated that the probiotic *L. plantarum* was successfully encapsulated in electrospun FOS/PVA/*L. plantarum* nanofibers with a selected prebiotic oligosaccharide as the wall material, and the electrospinning process had no obvious influence on the prebiotic effect of the screened oligosaccharide as well as on the viability of the loaded probiotic cells. Additionally, the viability of the probiotic cells encapsulated in the electrospun nanofibers was significantly enhanced by introducing FOS. Moreover, the encapsulation of probiotic cells in the above nanofibers significantly improved the resistance of the encapsulated cells to moist heat as compared to the free cells. This research exhibits an efficient approach for encapsulating probiotics, broadens the application of the prebiotic oligosaccharide, and provides a new idea

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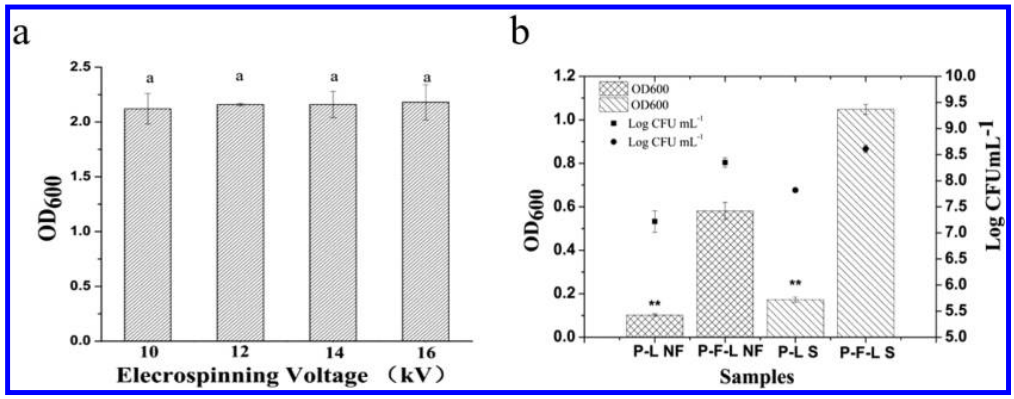

Figure 7. (a) The effect of applied voltage on the viability of the encapsulated *L. plantarum* cells. (b) The promotion effect of FOS added in electrospun fibers and solutions on the survivability of the encapsulated *L. plantarum* cells (P-L NF, PVA/*L. plantarum* nanofiber; P-F-L NF, FOS/PVA/*L. plantarum* nanofiber; P-L S, PVA/*L. plantarum* solution; P-F-L S, FOS/PVA/*L. plantarum* solution). Lowercase letters above the column indicate significant difference ($P < 0.05$) (**$P < 0.001$).

Table 3. Viability of Free Cells and Encapsulated Cells in Electrospun PVA/FOS/*L. plantarum* Nanofibers After Exposure to Moist Heat Treatments[a]

	no. of viable cells (log CFU/mL)			
samples	control	45 °C	60 °C	70 °C
free cells	9.47 ± 0.06 a	9.42 ± 0.01 a	4.57 ± 1.03 c	NP[b]
encapsulated cells	8.88 ± 0.34 a	8.84 ± 0.18 a	8.54 ± 0.16 a	6.17 ± 0.02 b

[a]Mean ± standard deviation with different lowercase letters which indicate significant difference ($P < 0.05$) for the samples under different temperatures. [b]Not probiotic enumeration (less than 6 log CFU/mL).

for the efficient preparation of effective and functional synobiotic supplements in the food industry.

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Notes

The authors declare no competing financial interest.

ABBREVIATIONS USED

FOS, fructooligosaccharides; GOS, galactooligosaccharides; IMO, isomaltooligosaccharides; PVA, poly(vinyl alcohol); SEM, scanning electron microscopy; TGA, thermogravimetric analysis; ATR-FTIR, attenuated total reflection-Fourier transform infrared spectroscopy; *L.*, *Lactobacillus*; *B.*, *Bifidobacterium*; MRS, de Man−Rogosa−Sharpe.

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