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# Protection mechanism of alginate microcapsules with different mechanical strength for *Lactobacillus plantarum* ST-III

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ABSTRACT

Alginate microcapsule is widely used to encapsulate and protect lactic acid bacteria (LAB). This study aims to design microcapsules with different mechanical strength and clarify their protecting mechanism for *Lactobacillus plantarum* ST-III under simulated digestive conditions through physical and physiological analysis. By controlling viscosity and gelling ability of alginate solution, a series of microcapsules with the same particle size (ca. 400 μm) and varying mechanical strength (5.4–51.9 N) were prepared. Cell survivals in simulated gastric juice (SGJ) and bile salts (BS) were found to be positively correlated with the mechanical strength of microcapsules. Diffusion experiments using different probes showed that the permeability coefficients of the microcapsules decreased with increasing mechanical strength. Moreover, membrane integrity and membrane fluidity of encapsulated LAB in SGJ were reduced to a less extent and tended to maintain at normal physiological values, with increasing mechanical strength. In contrast, cell membrane integrity was much more reduced (<4.0%) and membrane fluidity was markedly augmented in BS, regardless of the mechanical strength of microcapsules, indicating seriously damaged cells with abnormal physiological properties. These physical and physiological indexes together manifested the protection mechanism of alginate microcapsules with different mechanical strength for LAB.



## 1. Introduction

Probiotic lactic acid bacteria (LAB) are important microbial food ingredients, having multiple functions such as immune-modulation, enhancing barrier against pathogen adhesion, and alleviating antibiotic-associated diarrhea, irritable bowel syndrome and inflammatory bowel disease (Gareau, Sherman, & Walker, 2010). However, LAB are vulnerable to adverse conditions, such as acid, bile salts, heat, pressure and oxygen, leading to significant loss in cell viability (Tripathi & Giri, 2014). Microencapsulation is recognized as an effective way to enhance LAB survival and has been widely used in the food industry (Burgain, Gaiani, Linder, & Scher, 2011). Among various encapsulating materials, alginate was the most studied system for LAB due to its good biocompatibility, low immunogenicity and operational simplicity for gelation.

Capsule size is one of the key physical properties of alginate microcapsules. LAB cell viability in simulated gastric juice (SGJ) was shown to increase with increasing microcapsule size (Chandramouli, Kailasapathy, Peiris, & Jones, 2004). Mechanical strength is another important physical property of microcapsules that influenced LAB survivability under adverse conditions. Sandoval-Castilla et al. found that the viability of *Lb. casei* in yoghurt or SGJ was positively correlated with the mechanical properties of alginate beads (hardness, springiness, cohesiveness, and resilience) (Sandoval-Castilla, Lobato-Calleros, García-Galindo, Alvarez-Ramírez, & Vernon-Carter, 2010). Our previous studies showed that cell survivability in SGJ and bile salts (BS) was positively correlated with the mechanical strength of alginate microcapsules (Qu et al., 2016; Zhao et al., 2015). However, the limitation of the previous studies was the simultaneous variation of microcapsule size and mechanical properties. These two factors both contributed to an increase in LAB viability, thus complicating the

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determination of their respective contributions to the prospective effect of alginate microcapsules on LAB viability.

Limited knowledge has been available regarding the protection mechanism of alginate microcapsules for LAB. Cell viability seemed to be linked with the swelling/shrinking behaviors of alginate microcapsules in different digestive juices (Qu et al., 2016; Zhao et al., 2015), implying that microcapsule permeability may play a critical role in determining cell viability. Elucidation of the protection mechanism of alginate microcapsules needs the analysis of basic physiological properties of LAB cells such as membrane integrity, membrane fluidity etc. Intracellular pH and $NH_4^+$ concentration were reported to change significantly under acid stress (Zhang, Wu, Du, & Chen, 2012). Schwab et al. studied the relationship between probiotic survival and cell membrane properties (membrane integrity, membrane fluidity and membrane lateral pressure) during freeze-drying and storage, and found that cell survival with different protectants was correlated with the change of membrane properties (Schwab, Vogel, & Gänzle, 2007).

This paper aims to clarify the protection mechanism of alginate microcapsules for *Lactobacillus plantarum* ST-III under digestive conditions, by designing and controlling the physical properties of alginate microcapsules. Konjac glucomannan (KGM) was used to control the viscosity of alginate solution at the same level and oligoguluronate block (GB) was added to adjust the gelling ability of alginate solution. These allow the production of a series of alginate microcapsules with the same particle size but varying mechanical strength. KGM from the tuber of *Amorphophallus konjac* C. Koch has been consumed in Asia for centuries. It was reported to improve mice microflora and was regarded as a potential prebiotic (Chen, Fan, Chen, & Chan, 2005). Due to its large molecular weight, KGM is often used as a thickener to control the viscosity of food dispersions. GB was produced from alginate by selective acid hydrolysis (Simensen, Smidsr, Draget, & Hjelland, 2000). Due to its high guluronate content and low molecular weight, GB could be used to modulate the gelling kinetics and mechanical properties of alginate solution without changing the viscosity (Liao et al., 2015).

Using the well designed alginate microcapsules, the viability of encapsulated LAB cells in SGJ and BS is measured. Together with the analysis of permeability of alginate microcapsules and the physiological parameters of LAB (membrane integrity and membrane fluidity), the protection mechanism of alginate microcapsules is elucidated both from physical and physiological aspects.

## 2. Materials and methods

### 2.1. Materials

*L. plantarum* ST-III was provided by Bright Dairy & Food Co., Ltd (Shanghai, China). Encapsulating materials included sodium alginate (Manucol DM, FMC BioPolymer, Philadelphia, USA), KGM (Qingjiang Konjac Products, Wuhan, China), Nano-sized $CaCO_3$ (Zhenxin Reagent, Shanghai, China) and Span 80 (Xilong Reagent, Guangdong, China). Fluorescence agents for cell physiological properties included Live/Dead BacLight Bacterial Viability kit (Molecular Probes, Eugene, OR, USA) and Laurdan (Aladdin Reagent, Shanghai, China). Bile salt No. 3, pepsin (3000 U $mg^{-1}$), methylene blue (MB), vitamin $B_{12}$ ($VB_{12}$), FITC-dextran (10 kDa) and other chemicals were purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). Simulated gastric juice (SGJ) contained 3.2 g $L^{-1}$ pepsin and 9 g $L^{-1}$ NaCl solution at pH 2.0, while bile salts solution (BS) consisted of 10 g $L^{-1}$ bile salts at pH 6.8.

### 2.2. Preparation of oligoguluronate block

Oligoguluronate block (GB) was prepared according to a previous report (Liao et al., 2015). 20 g alginate was added into 200 mL HCl (0.3 mol $L^{-1}$), stirred overnight and then incubated at 95 °C for 5 h. The precipitate was collected from the acid-hydrolyzed solution by centrifugation (750 g, 15 min) and re-suspended in 200 mL HCl (0.3 mol $L^{-1}$). The procedure was repeated three times. The precipitate was then dissolved in 200 mL Milli-Q water and adjusted to pH 3.5 using 1 mol $L^{-1}$ NaOH. After overnight agitation, the precipitate was collected by centrifugation (750 g, 15 min) and washed with Milli-Q water (pH 3.5) for three times. The washed precipitate (GB) was dissolved in Milli-Q water (pH 7.0), and was filtrated through 0.45 μm membrane and freeze-dried.

### 2.3. Cell encapsulation

The cultivation of *L. plantarum* ST-III and cell encapsulation using emulsification/internal gelation were conducted as described previously (Qu et al., 2016). By addition of KGM and GB, a series of alginate microcapsules with different mechanical strength were designed (Table 1). The calcium/alginate monomer molar ratio ([Ca]/[M + G]), acid/calcium molar ratio ([H]/[Ca]) and acidification time were fixed at 0.67, 6 and 30 min, respectively. Take group E for example. The monomer (guluronic or mannuronic acid residue, $C_6H_{10}O_7$, $M_w = 194$) concentration of 30 g $L^{-1}$ sodium alginate and 2 g $L^{-1}$ GB was about 150 mmol $L^{-1}$ and 10 mmol $L^{-1}$, respectively, which thus required the addition of 106.7 mmol $L^{-1}$ $CaCO_3$ and 640 mmol $L^{-1}$ acetic acid.

20 mL alginate (with KGM or GB) solution was blended with $CaCO_3$ and 10 mL cell concentrate (about 10 log cfu). The cell mixture was emulsified in 70 mL soybean oil containing 0.7 mL Span 80, using a mechanical stirrer (IKA-Eurostar mixer, Staufen, Germany) at 300 rpm for 15 min. 20 mL soybean oil containing glacial acetic acid was added into the W/O emulsion and the mixture was stirred at 100 rpm for 30 min. Excessive phosphate buffer (0.1 mol $L^{-1}$, pH 7) was used to separate and wash microcapsules from oil phase. Microcapsules were harvested by centrifugation (10,000 g, 4 °C, 20 min) and stored at 4 °C for further analysis.

### 2.4. Physical properties of microcapsules

#### 2.4.1. Morphology and particle size measurements

As reported previously (Qu et al., 2016; Zhao et al., 2015), microcapsule morphology was observed by optical microscopy using BT-1600 image particle size analyzer (Bettersize Instruments Ltd, Dandong, China). Particle size was determined using Malvern Mastersizer 2000 (Malvern Instruments Ltd, Malvern, UK).

#### 2.4.2. Mechanical strength

Mechanical strength of the microcapsules was measured on Haake Rheostress 6000 rheometer (Thermo Scientific, USA) using a parallel-plate geometry (35 mm in diameter) at 25 °C (Qu et al., 2016; Zhao et al., 2015). A compression mode was applied and the normal force ($F_n$) during compression was recorded. $F_n$ at a gap distance of 0.15 mm was chosen to characterize the mechanical strength. Average value of $F_n$ from triplicate measurements was reported.

### 2.5. Cell survivals

#### 2.5.1. Encapsulation yield

The measurement of encapsulation yield (EY) was according to previous reports (Qu et al., 2016; Zhao et al., 2015):

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