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Protection of heat-sensitive probiotic bacteria during spray-drying by sodium caseinate stabilized fat particles

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ABSTRACT

The objective of this research was to investigate the possibility of co-encapsulating low melting point fat (LMF) with probiotic bacteria to minimize their heat damage during spray-drying. Three *Lactobacillus* isolates (LB1, S64 and K67) as potential probiotic bacteria were spray-dried in 10% w/v sodium caseinate (NaCas) in the presence of either LMF or vegetable oil as a control. Addition of LMF, but not vegetable oil, significantly increased the survival of isolate LB1 (from 15 % to 63 %) in the spray-dried powders. Differential scanning calorimetry (DSC) results showed that the melting enthalpy increased with the concentration of LMF, which is in accordance with the survival rate of LB1 after spray-drying. In addition, isolates LB1 and K67 co-encapsulated with LMF showed improved survival on NaCl-MRS agar compared to the control, indicating a reduction in their cellular damage. The current study demonstrated that LMF was an effective thermal protectant during spray-drying of probiotic bacteria.

1. Introduction

Probiotics, defined as “live microorganisms that, when administered in adequate amounts, confer a health benefit on the host” (FAO/WHO, 2002), have become increasingly popular during the last decade. As recommended by Shah and Ravula (2000), foods that contain such bacteria should contain at least 10^6 live microorganisms per g or mL at the time of consumption to produce therapeutic benefits. However, even though the foods satisfy such requirement before eating, the viability of probiotics from most commercial products is often questionable after they experience the low pH in the stomach and high bile salt conditions in the intestine (DeCastro-Cislaghi, Silva, Fritzen-Freire, Lorenz, & Sant'Anna, 2012; Lian, Hsiao, & Chou, 2003; Paéz et al., 2012). Providing probiotic living cells with a physical barrier (as so called “microencapsulation”) to resist adverse environmental conditions is therefore an approach currently attracting a considerable interest.

Among commonly used microencapsulation technologies, spray-drying is most suitable for large-scale industrial production because it is a continuous and rapid process with low cost and high reproducibility. However, during spray-drying, the probiotic cells may suffer from heat stress, dehydration, oxygen exposure and osmotic stress, thus leading to the damage of membranes, proteins, DNA and RNA (Meng, Stanton, Fitzgerald, Daly, & Ross, 2008; Paéz et al., 2012; Teixeira, Castro, Mohácsi-Farkas, & Kirby, 1997; Teixeira, Castro, Malcata, & Kirby, 1995). Lian, Hsiao, and Chou (2002) found that only 2.15% of *B. infantis CCRC 14633* survived during spray-drying in 10% (w/w) gum arabic. Golowczyc et al. (2011) found that 0.70 log CFU/mL of *Lactobacillus kefir 8348* was lost during spray-drying in 11% (w/v) reconstituted skim milk. Similar results of different degrees of cell death during spray-drying were also reported by other researchers (O'Riordan, Andrews, Buckle, & Conway, 2001; Picot & Lacroix, 2004). Several methods have been developed and showed various degrees of success in improving the probiotic survival after spray-drying. These include selection of thermal resistant strains (Picot & Lacroix, 2004), treatment of bacteria with mild heat before spray-drying (Paéz et al., 2012) and use of prebiotics or protectants such as granular starch, soluble fiber and trehalose (Burgain, Gaiani, Linder, & Scher, 2011).

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Low melting point fat (LMF), such as shortening and margarine, is a cheap food ingredient and is widely used in the production of cookies, cakes and fillings. The LMF transforms from solid to liquid phase rapidly at temperatures above its melting point, during which it absorbs considerable thermal energy. This phase transition has been observed for emulsions prepared using LMF as the core material using differential scanning calorimetry (DSC) (Maruyama et al., 2014; Palanuwech & Coupland, 2003; Thanasukarn, Pongsawatmanit, & McClements, 2004; Vanapalli, Palanuwech, & Coupland, 2002). At and below room temperature, LMF returns to its solid phase. We think this phase transition property could be applied favorably to microencapsulation of probiotics by co-encapsulation of LMF with probiotics via spray-drying. We hypothesized that the melting of LMF would absorb part of the heat energy during spray-drying, which would decrease the internal temperature of a particle, thus minimizing the heat shock to the probiotics. Consequently, this protective effect would minimize heat damage to the probiotics and improve their survival after spray-drying. To our best knowledge, the use of LMF as core material to protect probiotics during spray-drying has not been reported previously.

Sodium caseinate, with good film-forming property and emulsifying capacity, has already been used to encapsulate probiotic bacteria through spray-drying (Crittenden, Weerakkody, Sanguansri, & Augustin, 2006). The objectives of this study were to investigate the influence of LMF addition to the caseinate matrix on the survival of bacteria during spray-drying and its mechanism of protection.

2. Materials and methods

2.1. Materials

Sodium caseinate (NaCas) was purchased from Sigma–Aldrich Chemical Co., Ltd (St. Louis, MO, USA). Vegetable oil and low melting point fat (LMF) were obtained from UNICO Inc. (ON, Canada) and IOI Loders Croklaan Inc. (SansTrans™ 39, IL, USA), respectively. All glassware used in this study was sterilized at 121 °C for 15 min. The stains 4′, 6-Diamidino-2-phenylindole (DAPI) and fluorescein-isothiocyanate (FITC) were purchased from Sigma–Aldrich (St-Louis, MO, USA), and 9-diethylamino-5H-benzo [α]phenoxazine-5-one (Nile Red) from Kodak (Rochester, NY, USA).

2.2. Bacterial cell preparation

Lactobacillus zeae LB1, *Lactobacillus reuteri S64 and K67* were the isolates from chicken or pig intestines with the capacity to inhibit *Salmonella* or *Escherichia coli* infection in *Caenorhabditis elegans*, broiler chickens, and pigs (Wang et al., 2011; Yang et al., 2014; Yin et al., 2014; Zhou et al., 2014). The isolates from the stock cultures in 15% (v/v) aqueous glycerol at −80 °C were firstly cultured on de Man, Rogosa and Sharpe MRS agar (BD Institution, MD, USA) for recovery and single colony purification. To prepare the bacterial cultures for encapsulation, each isolate was sub-cultured twice in MRS broth with each at 37 °C for 24 h. A 12 h-grown fresh culture in MRS broth was then prepared. All the cultures were grown under anaerobic atmosphere (80% N_2, 15% CO_2 and 5% H_2). It is known that a probiotic culture in the stationary phase often has better heat resistance than in the exponential phase (Teixeira, Castro, & Kirby, 1994); thus the *Lactobacillus* isolates used in the present study were all harvested in the early stationary phase. Bacterial cells were harvested by centrifugation (Sorvall™ RC 6 Plus, Thermo Scientific Inc., MA, USA) at 4000 × g for 20 min (4 °C) and washed twice in sterile 0.85% (w/v) sodium chloride solution. The pellet was then re-suspended in the saline solution to obtain a suspension that contained approximately 10^{10} colony-forming unit (CFU)/mL. The bacterial suspension (10^{10} CFU/mL) was then stored at 4 °C and used on the same day.

2.3. Bacterial cell microencapsulation

LMF was preheated at 50 °C in a water bath to dissolve all crystals. Vegetable oil or LMF was then added into 100 mL NaCas solution (10% w/w, 40 °C) with different core to NaCas ratio (0, 0.25, 0.50, and 1.00 w/w). NaCas solution without vegetable oil or LMF was used as a control. The mixtures were coarsely mixed using a blender (Polytron® PT 10–35 GT-D, Kinematica Corporation, Switzerland) at 6000 rpm for 1 min (40 °C) and then recirculated through a high pressure homogenizer (Nano DeBEE, B.E.E. International Inc., MA, USA) at 3000 psi 3 times (40 °C). After placing the prepared emulsions at 0 °C overnight, *Lactobacillus* cultures were dispersed into the emulsions and stirred at 100 rpm for 10 min at 0 °C. The final solutions (10^9 CFU/g dry coating material) were then spray-dried in a laboratory scale spray dryer (ADL 310, Yamato Scientific America Inc., CA, USA). A constant inlet temperature of 170 °C and outlet temperature of 80 °C and a flow rate of 5 mL/min were used. Dried powder samples were then collected from the base of the cyclone and stored in tightly sealed sterile bottles at 4 °C.

2.4. Thermal tolerance of different bacteria

The thermal tolerance of bacteria was compared in NaCas solution (10%, w/w). Two 50 mL bottles containing 19 mL NaCas solution were placed in a water bath at test temperatures of 54, 57, 60, 63 and 66 °C. One of the bottles was used to monitor the temperature. When the desired temperature was reached, 1 mL of either LB1, S64 or K67 cell suspension was added to the second bottle. At selected intervals (between 30 s and 5 min), 1 mL aliquots were removed from the test bottle, serially diluted in MRS broth and plated on MRS agar for CFU counts. Enumeration was performed after 24 h of anaerobic incubation at 37 °C. The plating and enumeration were accomplished using the Eddy Jet Spiral Plater (Neu-tec Group, Farmingdale, NY, USA).

2.5. Survival rate

Bacterial cell viability was determined by the standard plate counting method. Spray-dried powders (0.5 g) were dispersed in 4.5 mL 0.2 M phosphate buffer (pH 7.0) and homogenized for 1 min at 4000 rpm (Polytron® PT 10–35 GT-D, Kinematica Corporation, Switzerland). Enumeration of cells was carried out by plating on MRS agar. Colony forming units (CFU) were enumerated manually after incubation at 37 °C for 24 h.

$$\textit{Survival rate}\ (\%) = \frac{\textit{CFU/g spray dried powder}}{\textit{CFU/g total solid in initial solution}} \times 100\%$$

2.6. Water content and water activity

Weighing dishes were first dried in an oven (105 °C) to a constant weight and then cooled in a desiccator containing silica gel. The weight of the empty dish was recorded (a), and approximate 3 g of powder was added, and the dish weighed again (b). The loaded dish was placed in the oven at 105 °C for 24 h, then cooled to room temperature in a desiccator and weighed again (c). The