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Survival of spray dried microencapsulated *Lactobacillus casei* ATCC 393 in simulated gastrointestinal conditions and fermented milk

Dimitra Dimitrellou [a, *], Panagiotis Kandylis [a], Tanja Petrović [b], Suzana Dimitrijević-Branković [c], Steva Lević [b], Viktor Nedović [b], Yiannis Kourkoutas [a]

[a] Applied Microbiology and Molecular Biotechnology Research Group, Department of Molecular Biology and Genetics, Democritus University of Thrace, Alexandroupolis, 68100, Greece
[b] Institute of Food Technology and Biochemistry, Faculty of Agriculture, University of Belgrade, Belgrade-Zemun 11080, Serbia
[c] Department of Biochemical Engineering and Biotechnology, Faculty of Technology and Metallurgy, University of Belgrade, Belgrade 11000, Serbia

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ABSTRACT

In the present study, the survival of spray-dried microencapsulated *Lactobacillus casei* ATCC 393 cells was evaluated under simulated gastrointestinal conditions, as well as during production and storage of fermented milk. Under simulated gastric juice and bile salts solutions, microencapsulation provided significantly improved protection compared to free cells. Furthermore, it retained a significantly higher number of viable *L. casei* compared to free cell system during fermented milk production. No significant differences in the physicochemical and sensorial characteristics of produced fermented milk with spray-dried *L. casei* cells were observed compared to the control and commercial samples, which is an important feature for consumers' acceptability. Importantly, after all *in vitro* tests, the number of microencapsulated *L. casei* cells remained over 6 log cfu g^{-1}, in contrast to free cells, which is the minimum required level for conferring a probiotic effect.

1. Introduction

Currently, a great interest exists in developing novel foods that contain probiotic microorganisms with numerous potential health benefits (Tripathi & Giri, 2014). It is essential for probiotic microorganisms to be characterized as safe for human consumption and therefore, the most widely used in probiotic foods are bacteria belonging to the genera *Lactobacillus* and *Bifidobacterium*, which are both naturally present in the human intestine (Tripathi & Giri, 2014). Among *Lactobacillus* sp., *Lactobacillus casei* is one of the most commonly used for applications in probiotic food products. More specifically, *L. casei* ATCC 393 is a well-studied strain with numerous potential probiotic (Monteagudo-Mera et al., 2012) and health promoting characteristics, such as removal of cholesterol (Lye, Rusul, & Liong, 2010), activity against cancer cell proliferation (Choi et al., 2006) and reduction of the risk of osteoporosis (Kim et al., 2009). *L. casei* ATCC 393 has been successfully used for the production of several dairy products like fermented milk (Kim et al., 2009; Sidira et al., 2010), cheese (Dimitrellou, Kandylis, Sidira, Koutinas, & Kourkoutas, 2014b) and yogurt (Dimitrellou, Kandylis, & Kourkoutas, 2014a; Sidira et al., 2013).

Probiotic cells should be able to maintain their viability and stability throughout storage, and they must survive the harsh conditions commonly encountered in the gastrointestinal tract of humans, in order to deliver the health benefits (Kim et al., 2008). However, most of the probiotic strains, including lactic acid bacteria, are unable to fulfill the above requirement (Li, Chen, Sun, Park, & Cha, 2011; Rajam, Karthik, Parthasarathi, Joseph, & Anandharamakrishnan, 2012).

One method that has been proposed to overcome the low viability problems of probiotics is microencapsulation which can provide protection to sensitive cells during food processing and storage and ensure target delivery in the gastrointestinal tract (Anal & Singh, 2007; Champagne & Fustier, 2007; Manojlović, Nedović, Kailasapathy, & Zuidam, 2010; Petrović, Nedović, Dimitrijević-Branković, Bugarski, & Lacroix, 2007). Microencapsulation of probiotics has been carried out by several methods, such as spray-drying, fluid bed coating, spray-chilling/cooling, extrusion and emulsion and freeze- or vacuum drying (Nedović, Kalusević, Manojlović, Lević, & Bugarski, 2011; Nedović, Kalušević,

* Corresponding author.
E-mail address: dimitrellou@gmail.com (D. Dimitrellou).

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Manojlović, Petrović, & Bugarski, 2013). Furthermore, storage of probiotic microorganisms for a long period of time prior to their use is often required by the food industry and thus culture preservation is an industrial necessity (De Giulio et al., 2005).

Today, the most commonly used method for the drying of bacterial cultures is freeze-drying, mainly due to its low-temperature drying conditions (Paéz et al., 2012). An interesting alternative is spray-drying, leading up to 80% reduction in operational and manufacturing costs (Santivarangkna, Kulozik, & Foerst, 2007) and allowing continuous operation and thus yielding higher production rates (Zamora, Carretero, & Pares, 2006). In addition, the obtained powder is stable and easy for application. Yet, the requirement of high temperatures leads to low bacterial viability, which is the main drawback (Ananta, Volkert, & Knorr, 2005). However, several studies have been carried out evaluating spray-drying as a promising method for protection of microbial cells against the adverse conditions to which they can be exposed during processing, storage and through the gastrointestinal transit (Arslan, Erbas, Tontul, & Topuz, 2015; Bustamante, Villarroel, Rubilar, & Shene, 2015; Fritzen-Freire, Prudêncio, Pinto, Muñoz, & Amboni, 2013; Pinto et al., 2015; Rajam & Anandharamakrishnan, 2015).

In the present study, spray-drying was employed for microencapsulation of *L. casei* ATCC 393 cells with skim milk as a carrier. The aims were to study the viability of free and microencapsulated *L. casei* ATCC 393 cells under simulated conditions of gastrointestinal transit, as well as in fermented milk product. In addition, the effect of microencapsulation on the physicochemical, microbiological and sensorial characteristics of the produced fermented milk was investigated. To the best of our knowledge, this is the first report concerning evaluation of *L. casei* ATCC 393 in spray-dried form under simulated gastrointestinal conditions and during fermented milk production and storage, providing a complete research assay for efficient industrial exploitation.

2. Materials and methods

2.1. Strains

L. casei ATCC 393 (DSMZ, Braunschweig, Germany) was used as probiotic strain. The culture was prepared, as previously described in Petrović et al. (2012) and then used to inoculate the feed media.

The thermophilic starter, CH-1 (Chr. Hansen, Hørsholm, Denmark) consisted of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* spp. *bulgaricus* in a freeze-dried form was also employed in the present study. Prior use, it was activated by adding a 50 U sachet to 500 mL of sterile reconstituted skim milk (140 g L^{-1}) and agitated for 15 min to achieve an homogenous culture.

2.2. Microencapsulation procedure and encapsulation yield

Briefly, spray-dried feed media was consisted of reconstituted skim milk (200 g L^{-1}), prepared by dissolving the carrier in distilled water and autoclaving at 105 °C for 5 min. A mini spray-dryer (B290 Büchi, Switzerland) was used to produce samples at a constant air inlet temperature of 170 °C, while the outlet air temperature was maintained at 80–85 °C, in order to obtain powders with around 4% moisture content. The feed solution was atomized in a drying chamber using a two-fluid nozzle.

Per cent of survived bacteria were calculated as described in Petrović et al. (2012).

The encapsulation yield (EY) was calculated using the following equation (Maciel, Chaves, Grosso, & Gigante, 2013): $EY = (\log N/\log N_0) \times 100$, where N is the number of viable cells (cfu g^{-1}) in the powder and N_0 is the number of viable cells (cfu g^{-1}) in the encapsulating material before drying.

Water activity was determined at 22 °C using Testo 650 (Germany) instrument.

2.3. Survival of microencapsulated L. casei cells

To determine the viable counts of the microencapsulated *L. casei*, 0.1 g of spray-dried samples were suspended in 9.9 mL ¼ Ringer's solution and stirred for complete homogenization. The colony forming units (cfu g^{-1}) were determined by anaerobic plating on de Man, Rogosa, and Sharpe (MRS) agar plate and incubating at 37 °C for 72 h. The plating procedures were carried out in triplicate.

2.4. Survival of microencapsulated L. casei cells in simulated gastric juice

A simulated gastric solution containing NaCl was prepared by suspending pepsin (3 g L^{-1}) in saline (5 g L^{-1} NaCl) and adjusting the pH to 2.0 or 3.0 with 5 mol L^{-1} HCl solution (Li et al., 2011). After sterile-filtration through a membrane (0.45 μm, Gelman Science, Ann Arbor, MI, USA), samples of 0.1 g of microencapsulated *L. casei* or 0.1 mL of the free cell suspension were placed separately in test tubes containing 9.9 mL simulated gastric pH solution and incubated at 37 °C. After 0.5 h, 1 h, 2 h and 3 h of incubation samples were taken and immediately used for the enumeration of viable cells, as described previously.

2.5. Survival of microencapsulated L. casei cells in simulated bile conditions

Tolerance of microencapsulated *L. casei* to simulated bile salt was carried out with the same procedure as described for low pH tolerance, but 9.9 mL of bile salt solution containing 5 or 10 g L^{-1} bile salts were used (Li et al., 2011). Triplicate samples were withdrawn after incubation for 0, 3 and 6 h and cell counts of free and microencapsulated *L. casei* were enumerated on MRS agar.

2.6. Fermented milk production with free or microencapsulated L. casei

2.6.1. Procedure

Pasteurized bovine milk (37 g L^{-1} fat) was tempered at 37 °C and the microencapsulated (1 g dried L^{-1}) or free *L. casei* cells (4 g wet L^{-1}) were added. After 30 min, all samples were inoculated with the activated CH-1 culture (0.3% v/v). Milks were fermented at 37 °C until pH value of 4.6 was obtained. Thereafter, the fermented milks were cooled to 15 °C in ice water and stored at 4 °C for 28 days. For comparison reasons, milk fermented only with yogurt culture CH-1 was also produced (control sample).

2.6.2. Determination of culture viability

Fermented milk samples were added to ¼ Ringer's solution and the appropriate serial dilutions were prepared. *S. thermophilus* was enumerated using M–17 agar supplemented with lactose at 45 °C, *Lactobacillus bulgaricus* was enumerated using MRS agar, adjusted to pH 5.2 at 45 °C and *L. casei* was enumerated using lithium propionate MRS (LP-MRS) agar (lithium chloride 2 g L^{-1} and sodium propionate 3 g L^{-1}) at 37 °C (Vinderola & Reinheimer, 2000).

2.6.3. pH and titratable acidity of fermented milks

pH of fermented milks was measured using a pH-meter and titratable acidity was determined by titration using 0.1 mol L^{-1} NaOH and phenolphthalein as an indicator and expressed as g of lactic acid per 100 g fermented milk.