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Effect of microencapsulation on viability and survival in simulated gut conditions of probiotic bacteria

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Abstract

Health benefits of probiotic bacteria led to their increasing use in different food and pharmaceutical products; however their viability is low in these products. Microencapsulation of probiotic bacteria has been investigated to improve probiotic's survival in foods and gastrointestinal environment. In this study Lactobacillus paracasei subsp. tolerans *isolated from human intestinal microbiota and identificated by 16S rDNA gene sequencing, was microencapsulated by spray drying. These results demonstrate that microencapsulation provided better protection at simulated gastric and intestinal conditions compared to free cells.*

Keywords: probiotic, spray drying, viability, storage, size distribution

1. Introduction

Probiotics were defined by the Food and Agriculture Organization/WHO as "live microorganisms which when administered in adequate amounts confer a health benefit on the host". Probiotics exert their beneficial effects through these mechanisms: interference with potential pathogens, improvement of barrier function, immunomodulation and production of neurotransmitters (SANCHEZ et al., [1]). Probiotics and lactic acid bacteria (LAB), which are typically associated with the human gastrointestinal (GI) tract (KIM et al., [2]), have been reported to suppress the growth of pathogens (QUIGLEY, [3]) and have been demonstrated the immunity-enhancing effect (VANDAMME et al., [4]). Genera of LAB include, among others, *Lactococcus*, *Enterococcus*, *Oenococcus*, *Pediococcus*, *Streptococcus*, *Leuconostoc* and *Lactobacillus*, they produce lactic acid by fermentation. The genus *Lactobacillus* is the largest group with over 100 species and subspecies (LASLO et al., [5]). LAB are classified into two groups according to their hexose metabolic pathways: homofermentative, where lactic acid is the main end-product and heterofermentative, which produce other end-products such as acetic acid, carbon dioxide or aroma compounds, in addition to lactic acid (NURAIDA, [6], ZAROUR et al., [7]).

Species of lactobacilli survive poorly under simulated gastrointestinal conditions (VANDAMME et al., [4], SABIKHI et al., [8]), however it would be important to reach the small intestine and colon, where they exert their effects. The physical protection of probiotics by microencapsulation is a new approach to improve the probiotic survival under different growing conditions (KRASAEKOOPT et al., [9]).

The safety of microcapsules is important when live cells are intended for use in the intestinal system by oral administration. This is because the live cells must be protected during the encapsulation process and microcapsules must reach the large intestine and the survival of the live cells during their passage must be ensured. The membrane of microcapsule must be

provided with sufficient permeability for nutrients, secretion and excretion products, to pass through and to prevent the entry of molecules which could destroy the encapsulated bacterial cell (ARIFUL et al., [10], OUYANG et al., [11]).

Spray drying technique is considered as a good long-term microencapsulation, preservation method especially for probiotic cultures. As stated out earlier it has been investigated as a means of stabilizing probiotic bacteria in foods. Spray drying is the most popular and cost effective process known to dry bacteria while keeping their viability. This process is readily available, easy to operate and energy efficient (CHAVEZ and LEDEBOER, [12]).

Spray drying is the most commonly used microencapsulation method in the food industry, is economical and flexible, produces a good quality product (PEIGHAMBARDOUST et al., [13]). The advantage of the process is that it can be operated continuous, the disadvantage is that the high temperature (~150°C) used in the process may affect bacterial survival. For the encapsulation of viable bacterial cells, the materials used as excipients should be gentle and non-toxic. The most commonly used material for microencapsulation of probiotic bacterial cells is alginate (KAILASAPATHY et al., [14]).

The aim of our study was to enhance viability of *Lactobacillus paracasei subsp. tolerans* isolated from human microbiota by encapsulation in maltodextrin-alginate and inuline-alginate microcapsules, furthermore to decipher the effect of co-encapsulation with prebiotics (maltodextrine and inuline). Considering the preliminary results it should provide additional protection to the bacterial cells (MIZIELIŃSKA et al., [15]).

2. Materials and Methods

2.1. Microorganism and its maintenance

A pre-culture of strain *Lactobacillus paracasei subsp. tolerans* B-33 in 200 ml of MRS broth was incubated at 37°C for 48 h, the cultured broth was centrifuged at 5000 rpm (d=12 cm) for 20 min. The resulting pellet was resuspended in 500 ml MRS broth and cultivated for 48 h, at 37°C. Before spray-drying cultured broth was centrifuged at 5000 rpm (d=12 cm) for 20 min and the pellet was resuspended in 10 ml of 8 g/l NaCl solution.

2.2. Counting of bacteria

Viable bacteria were counted in carrier solution prior to spray drying and in 0.1 g of powder after drying and during storage. Samples were serially diluted in triplicate from 10^{-1} to 10^{-5}, plated on MRS agar and incubated for 48 h at 37°C. Results were expressed in CFU (colony forming units)/g product (CHAVEZ and LEDEBOER, [12]).

2.3. Microencapsulation of the probiotic microorganisms

2.3.1. Preparation of the suspension for drying

Bacterial suspension was mixed with sterilized Ringer solution, in 1% proportion, this suspension was agitated with magnetic stirrer. Separately sodium alginate (2%) (Sigma-Aldrich) was mixed with maltodextrine, respective inuline (12%) (Sigma-Aldrich), this mixture was homogenized well, contrary in case of making suspension with water, will form clusters of alginate, hard to dissolve subsequently. The obtained mixture is dissolved in 30 ml of 8 g/l NaCl solution, to this suspension was added the bacterial suspension under strong stirring. It was obtained a viscous suspension, which is cooled to 4°C for 1-24 h to remove bubbles (FLOREA, [16]).

2.3.2. Encapsulation technique

The spray-drying process of the probiotic bacteria was undertaken in a laboratory-scale spray dryer (SonoDry 750, Sonotek, USA), following the user's manual. Briefly, the feed solution was ultrasonical atomized (120 kHz ultrasonic nozzle, 2 kW) in a drying chamber at constant flow rate: 2.5 ml/min, air temperature: 130°C, and flow rate 80 m^3/h. The dried powder was collected in a cyclone separator system.

2.4. Evaluation of microcapsules

Depolymerization is a technique used to break the microcapsules and release completely the organisms into the solution before evaluating their viable count (SABIKHI et al., [8]). 0.1 gram of the microencapsulated beads was mixed in test tubes containing 5 ml of depolymerization solution (28 ml of 0.2 M solution NaH_2PO_4 and 72 ml of 0.2 M Na_2HPO_4 adjusted to 200 ml volume with distilled water, pH 7.1±0.1, sterilized).

After incubation at 37°C for 10 min, the mixture was vortexed at high speed (2500 rpm) for breaking the polymer formed and releasing completely the encapsulated culture into the buffer solution. The released cells were enumerated using MRS agar media by incubating the samples for 48 h at 37°C (SABIKHI et al., [8]).

2.5. Effect of gastric and intestinal juices on the viability of encapsulated cells

Gastric juice is prepared by regulation of pH at 2.0 value with cc. hydrochloric acid or with sterile solution of 0.1M sodium hydroxide, using a pH-metre. Intestinal juice is prepared by resolving in sterile sodium chloride solution (0.5 w/v%) bile salts (0.5 w/v%) (Merck) and pancreatin (1 g/l) (Merck), pH is regulated at 8.0.

0.2 ml of bacterial cells suspension was transferred in a sterilized 2 ml Eppendorf tube, this suspension was mixed with 0.3 ml sterile solution of NaCl (0.5 w/v%) and 1 ml of gastric juice. This mixture is vortexed for maxim 10 s and incubated at 37ºC. Viability of strains in gastric juice is determined by inoculation on MRS media after different periods of incubation (0 min, 90 min), the samples are incubated for 48 h at 37ºC. After the incubation was determined the number of CFU/ml suspension.

The determination of probiotic cells viability in intestinal juice was performed in a similar way, as described hereinafter. 0.2 ml of bacterial cells suspension was transferred in a sterilized Eppendorf tube and was mixed with 0.3 ml sterile solution of NaCl (0.5 w/v%) and 1 ml of intestinal juice. The mixture obtained was vortexed for maxim 10 s and incubated at 37ºC for 120 minutes. Viability of strains in simulated intestinal juice is determined by inoculation on MRS media immediately after vortexing - for initial number of CFU/ml - and after 120 minutes of incubation to determine the final CFU/ml. The samples are incubated for 48 h at 37ºC and after incubation the number of CFU/ml was determined.

The evaluation of the viability of encapsulated cells: 0.1 g of encapsulated bacterial cells was transferred in a 10 ml sterile Falcon tube and mixed with 3 ml 0.5 w/v% sterile sodium chloride solution, 10 ml simulated gastric fluid, and10 ml of intestinal juice, respective. This mixture is vortexed for maxim 10 s and incubated at 37ºC. Viability of strains is analysed by determination of CFU/ml after 90 min of incubation in gastric environment, and 120 min of incubation in intestinal environment, respectively. Samples were inoculated on MRS agar solid media, CFU/ml was numbered after an incubation time of 48 h at 37ºC.

3. Results and discussion

3.1. Size distribution of microcapsules

Particle size distribution was measured by Mastersizer 2000 (Malvern). This instrument uses the technique of laser diffraction to measure the size of particles by measuring the intensity of light scattered as a laser beam passes through a dispersed particulate sample. This data is then analyzed to calculate the size of the particles that created the scattering pattern.

The average size (d 0.5) of the obtained microcapsules was 46.494 μm in case of microcapsules obtained from alginate-maltodextrine, respective 41.713 μm at alginate-inulin microcapsules (Figure 1.).

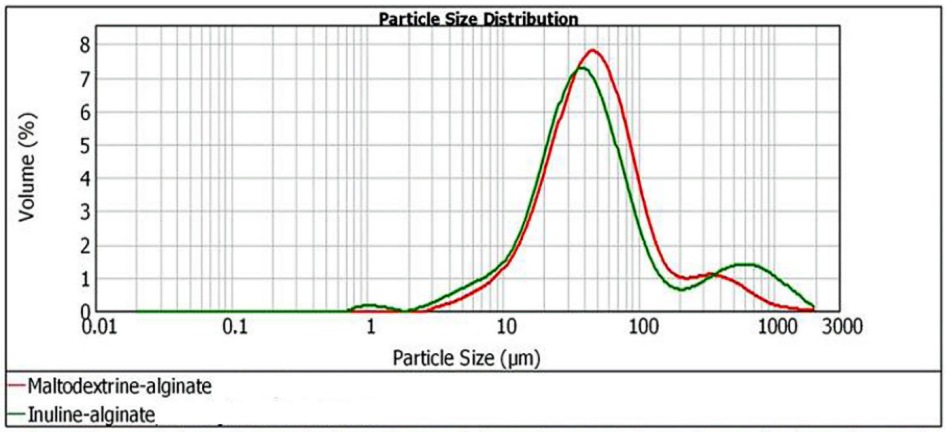

Figure 1. Particle size analysis of microcapsules.

3.2. Survival during the encapsulation process

High temperature of spray drying (130ºC) process affects bacterial cells viability. Initial cells number (CFU/ml) was calculated before spray drying by plate count method on MRS agar media. After the mixture of microcapsules containing bacterial cells and phosphate buffer was vortexed at high speed to release the encapsulated cells into the buffer, the CFU/g product was determined from the suspension obtained. Initial and final CFU/ml were compared to determine survival rate in this process.

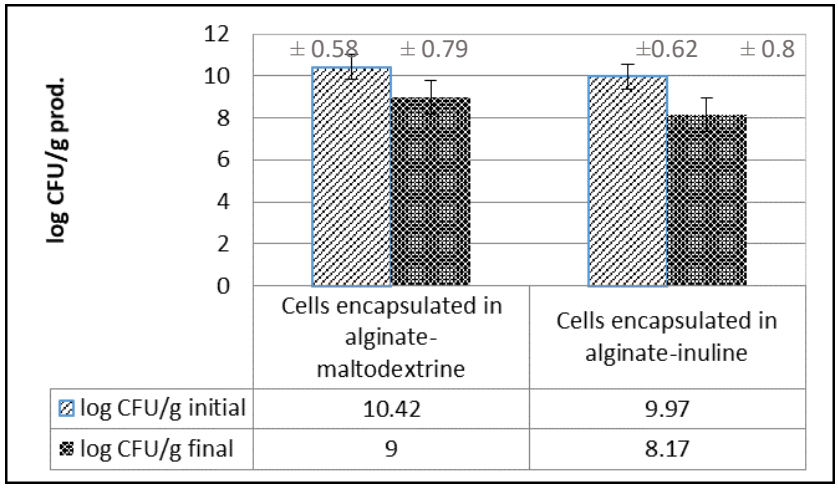

Figure 2. Survival during spray drying.

Bacterial cells encapsulated in alginate and maltodextrine (ballast material) have a survival rate of 0.075% during spray drying process, when bacterial cells were encapsulated in alginate and inuline (ballast material) survival rate was better (1%), but still the number of CFU are higher than 10^6/g product in both cases. KAILASAPATHY [14] considered that products sold with any health claims meet the criterion of a minimum $1*10^6$ CFU/g probiotic bacteria to achieve the therapeutic benefits.

3.3. Viability of encapsulated and non-encapsulated bacterial cells in gastrointestinal conditions *in vitro*

In order to exert beneficial health effects, probiotic microorganisms should resist the stressful effect of the low pH in stomach and bile salts in intestine (KIM et al., [2]). Survivals of free and encapsulated bacterial cells were detected after exposure to gastric and intestinal juices. The results expressed are showed in Figure 3.

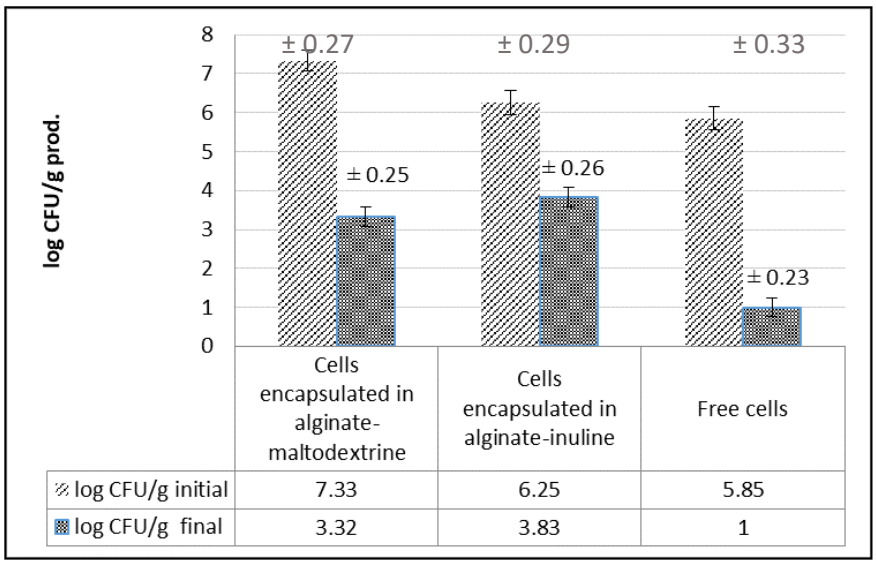

Figure 3. Survival of encapsulated and non-encapsulated cells in conditions of digestive system *in vitro*.

After exposure to acidic and intestinal conditions, survival rate of native cells is in range of 0.0001%, microencapsulated in alginate and maltodextrine (ballast material) survived in greater number (loss of viability is 4 log unit), that demonstrates that encapsulation may be effective to increase the survival of bacterial cells in gastrointestinal conditions. Cells encapsulated in alginate and inuline (ballast material) showed a better survival rate, about 0.328% (loss of viability is 2.4 log unit). These findings agree with earlier reports (SABIKHI et al., [8], MIZIELIŃSKA et al., [15]) and demonstrate that microencapsulated lactic acid bacterial cells in alginate beads survived better after incubation in simulated gastric and intestinal juices.

3.4. Survival during storage

The obtained product was stored at a relative humidity of 1% at 25ºC, the relative humidity was maintained constant by storing the powder in hermetically closed jar above $CaCl_2$. Number of CFU/g product was determined during five weeks of storage, once every week (in triplicate). The viability loss is minimal (0.1 log unit), results show high survival rate (90%) during storage of 5 weeks at mentioned conditions (Figure 4.). After ANANTA et al. [17] for a shelf-life period of 1 month a reduction of 0.25 log unit at 25ºC is occurred, high storage stability of probiotic bacteria at non-refrigerated temperatures shows sufficient protection.

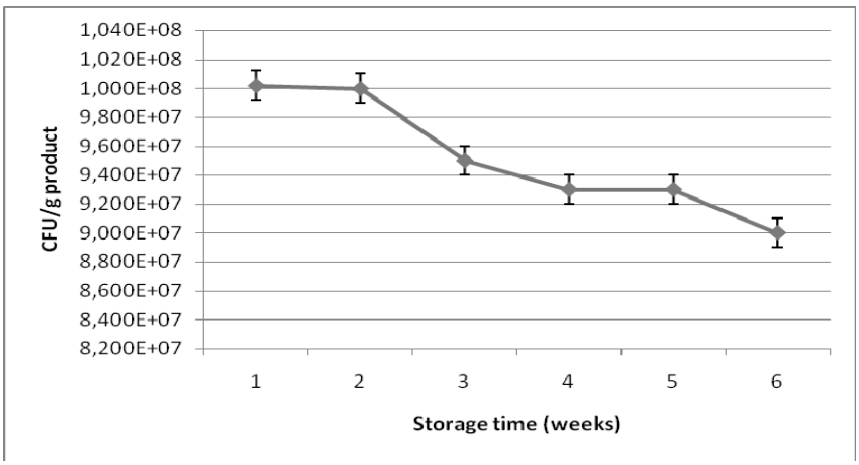

Figure 4. Viability of microencapsulated bacteria during storage.

4. Conclusion

The obtained microcapsules with an average diameter of 46.494 μm (in case of alginate-maltodextrine), respective 41.713 μm (alginate-inuline microcapsules), contained a lactobacilli CFU number between 10^6-10^8 /g product, this number is higher than $1*10^6$ CFU/g product which was considered the minimal number in probiotic products (KAILASAPATHY, [5]), this value remains above 10^6 after a storage of 6 weeks. The process of microencapsulation due to the high temperature of the process of spray drying, affected the viability of lactobacilli, the loss of CFU is near 2 log units. The comparation of the viability of encapsulated and non-encapsulated bacterial cells in gastric conditions showed that encapsulation provides protection for probiotic cells. Encapsulated cells surviving rate in gastric conditions are better when probiotic cells are encapsulated in alginate and inuline: viability loss is 2,4 log units/g product, better than in case of free cells (viability loss is 5 log units/g product). As a consequence: alginate and inuline should be used as encapsulation materials to enhance the probiotic effect of live microbial dietary supplements.

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