O3-1	**Effect of digestive processes on encapsulated lactic acid bacteria.**	

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INTRODUCTION AND OBJECTIVES

The positive impact of lactic acid bacteria on the human gastrointestinal tract was first observed in the beginning of the 20th century. In the following years it was proved that bacteria originating from intestine stand a far better chance of survival in human gut and delivery of the desired positive effect.

Nowadays, probiotic bacteria are incorporated into a large variety of food to provide health benefits to the host organism. Therefore, research is conducted all over the world to produce the highest possible yield of probiotic bacteria administration to their colonization site (Anal 2007, Kim 2008). One of the solutions might be microencapsulation – a process first developed in 1993.

Microencapsulation is a technique of microorganisms immobilization – one of the great breakthroughs of the previous century (Park 2000). The idea of targeted delivery and controlled release is currently becoming more and more popular in various branches of industry. Microcapsule is composed of a semi-permeable membrane composed of biopolymers with a liquid core allowing for floating of microorganisms within the sphere (Champagne 2007). In this research a double-layer microspheres were used, composed of alginate cross-linked with calcium ions and further hardened with chitosan. The choice of encapsulation method was made basing on the highest efficiency of the process and uniform size distribution of the obtained carriers.

Finally, two probiotic bacteria strains were chosen: *Lactobacillus casei* 0900 and *Lactobacillus paracasei* 0919 and submitted for their viability assessment in a GI tract simulator, both in a "free" form and in microcapsules to confirm the protective role of microspheres in harsh environment.

MATERIALS AND METHODS

GITS

Gastrointestinal tract simulator (Sumeri 2008) was set up in an anaerobic atmosphere provided by nitrogen and on a rotary shaker (50 rpm) with an incubator (37 °C).

1. Stomach (optionally with an addition of pepsin and trypsin)
    a. Empty stomach: 25 ml of 0.1 M HCl at pH 2.0
    b. 50 ml addition of "food" (LAB: 10^7-10^9 CFU/ml)
    c. Titration to pH 3.0 with 1 M HCl at flow rate 20 mmol/h
2. Passage from stomach to duodenum
    a. Neutralization to pH 6.0 with 1 M $NaHCO_3$ at flow rate 4.5 ml/min
3. Duodenum
    a. Preparation of 4 %(w/v) solution of bile salts
    b. Addition of bile salts up to an 0.4 %(v/v) concentration in the simulator at a flow rate 4.0 ml/min
    c. 30 min incubation with bile salts
4. Jejunum and ileum
    a. 10 times dilution with MRS (simulation of bile salts adsorption) at flow rate 6.0 ml/min
    b. Simultaneous titration with to pH 6.5 with 1 M $NaHCO_3$ (if necessary)

Lactic acid bacteria viability

The viability of bacteria was assessed by the plate count method. Series of bacterial suspensions dilutions were pour-plated with MRS medium-agar and colony-forming units determined from plates containing 30-300 colonies.

For microencapsulated probiotic bacteria the first dilution was made in 0.2 M phosphate buffer and shaken out on a reciprocating shaker for 3 hours before further dilutions.

For each survival rate calculation, the altering volume of the fluid was taken into account by the following formula:

$$LAB = \frac{CFU_x \times V_x}{CFU_{initial} \times V_{initial}}$$

Where:
CFU_x – colony-forming units at a given digestion stage
V_x – volume of fluid in the simulator at a given digestion stage
$CFU_{initial}$ – colony-forming units in "food"
$V_{initial}$ – volume of "food"

RESULTS AND DISCUSSION

The experiments described in the previous section were performed in triplicate for both strains: *Lactobacillus casei* 0900 and *Lactobacillus paracasei* 0919. The obtained results confirm the protective properties of alginate-chitosan microspheres, especially for the strain *Lb. casei* 0919, as illustrated in Figure 1 and Figure 2.

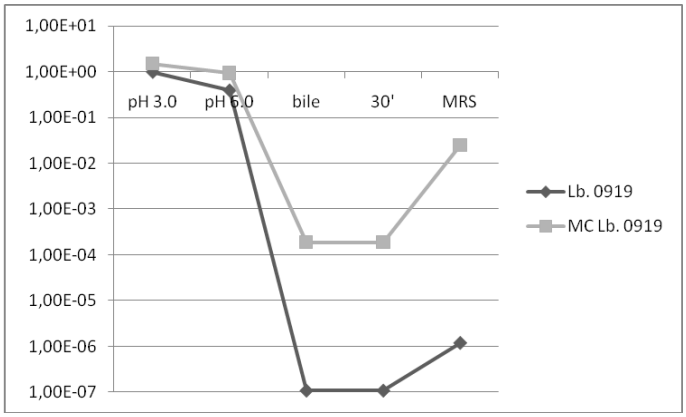

Figure 1 : Digestion of encapsulated and "free" *Lb. casei* 0900

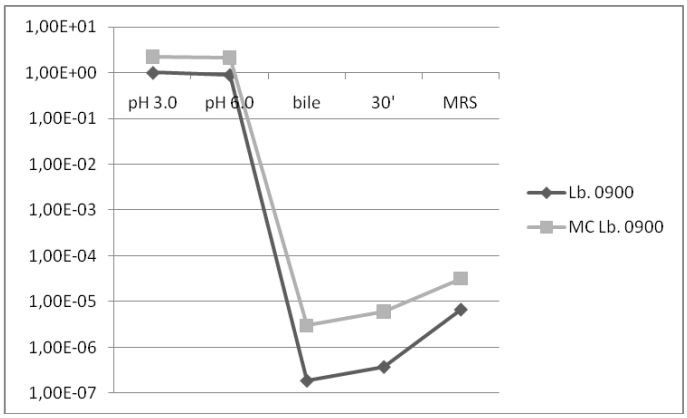

Figure 2 : Digestion of encapsulated and "free" *Lb. paracasei* 0919

Analyzed strains, both microencapsulated and "free", are resistant to low pH in the stomach and duodenum simulator. The situation changes when bile salts are incorporated in the solution. In the case of *Lactobacillus casei* 0900, the viability of bacteria drops by 5 and 7 orders of magnitude for encapsulated and "free" bacteria respectively. Encapsulated *Lb. paracasei* 0919 seems more resistant to bile salts, since its viability dropped only by 3 orders of magnitude. In comparison, 'free' *Lb. paracasei* 0919 declined by 6. For both strains, the 30 minutes incubation period had no influence on viability.

What is more, for all 4 systems examined in GITS, growth recovery was observed during the dilution with MRS stage. This fact confirms that the bacterial ability to multiply was preserved during the whole passage through the simulator.

CONCLUSIONS

In conclusion, alginate-chitosan microspheres significantly improve the viability of probiotic bacteria during the passage through the human gastrointestinal tract.

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