Viability of probiotics exopolymers beads exposed to specific *in vitro* conditions

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INTRODUCTION

Encapsulation can improve the survival of probiotics during their storage in foods (Adhikari, 2000; Adhikari, 2003; McMaster, 2005) and also their survival in the gastrointestinal tract (Chandramouli, 2004; Iyer, 2005; Lian, 2003). Most of the researches use alginate beads due to its easy handling (Kailasapathy, 2002). As our main work field is microbiology, the use of microbial based polymers for this purpose was our aim and that is why a mixture of gellan gum and xanthan gum has been chosen for our beads preparation. In order to reduce the bead diameter, an evaluation of beads preparation by using the dropping method with the application of an electrostatic potential between the needle and the collecting solution was previously performed (Jiménez-Pranteda, 2008). Once the method was validated, a study of the survival of encapsulated and non-encapsulated bacteria in simulated gastrointestinal conditions has been carried out. Also a comparison between two different strains of probiotics has been performed in each part of this research.

MATERIAL AND METHODS

Microencapsulation of probiotics
1 mL of a probiotics suspension (Jiménez-Pranteda, 2008) was added to 20 mL solution of 1% Xanthan gum + 0.75% Gellan gum. The suspension was extruded using an electrostatic droplet generator in a 0.1 M Calcium Chloride solution. The electrostatic droplet generator consists in a syringe pump equipped with a syringe, a needle and a high voltage generator connected to the needle and on the recovery solution (Poncelet, 2000). The electrostatic potential applied was 6.5 kV and the flow rate was 120 ml/h.

Cell enumeration was determined by counting plate method after bead dissolution with 0.05M phosphate buffer, pH=7. The medium used was MRS agar and incubations were performed at 37ºC in every case, 24 hours and 5% CO_2 with lactobacilli strain and 48 hours and anaerobic atmosphere with *Bifidobacterium* strain.

Survival of encapsulated and non-encapsulated bacteria in simulated gastrointestinal conditions
1 g of capsules or 100µL of probiotic concentrated in distillated water were added to 9 mL of MRS liquid medium formulated as described in Table 1. After contact, beads and free cells were washed with peptone water 0.5%, pH=6.5. Cell enumeration of encapsulated and free bacteria as well the supernatant of the beads tubes were made using plate technique on MRS agar as previously described.

Bacterial growth in a conditions range (Table 1) has been tested by optical density (λ=550nm) variation in order to select the optimum conditions for our work (Table 2).

pH	2, 3, 4, 5, 6, 7
Pepsin	0.01%, 0.02%, 0.03%, 0.04%
Pancreatin	0.1%, 0.2%, 0.3%, 0.4%
Bile	0.5%, 1%, 2%, 3%, 4%, 5%

Table 1. Conditions tested at λ=550 nm

Conditions	pH	Enzymes	Duration
Simulated gastric juice	3.5	0.03% pepsin	2 hours
Simulated intestinal juice	6.5	0,2% pancreatin	2 hours
Bile solution	6.5	3% bile	2-24 hours

Table 2. Simulated gastrointestinal conditions and sampling times.

RESULTS

Bacterial survival through the encapsulation method has been previously tested (Jiménez-Pranteda, 2008), getting a lower survival in comparison with non-electrostatic application method but a noteworthy reduction of size. Capsules are round shape (Fig.1) and mean diameter of 1.9±0.4mm.

Fig. 1% Xanthan gum + 0,75% Gellan gum beads with probiotics

As it is seen in Fig.1-2, the survival not only depends on the encapsulation process but also, and specially, on the bacterial strain.

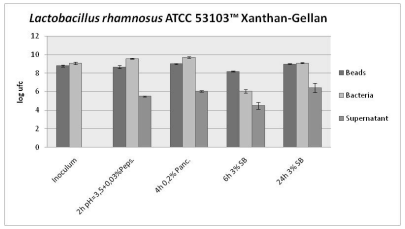

Figure 2. Gastrointestinal survival of *Lactobacillus rhamnosus* ATCC 53103™ encapsulated in 1% Xanthan gum + 0.75% Gellan gum and non-encapsulated.

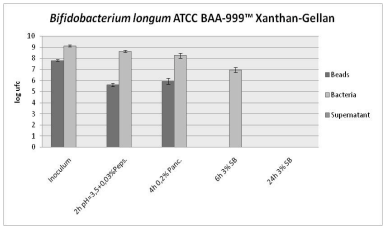

Figure 3. Gastrointestinal survival of *Bifidobacterium longum* ATCC BAA-999™ encapsulated in 1% Xanthan gum + 0.75% Gellan gum and non-encapsulated.

There are less concentration oscillations when using the lactobacilli encapsulated rather than lactobacilli non-encapsulated. It is also noteworthy that the supernatant bacterial concentration belongs to each condition separately because of it is discarded for bead washing after each condition residence. Bile salts reduce drastically the bacterial concentration in non-encapsulated lactobacillus during the initial 2 hours exposition (3 $\log_{10}$CFU) but the concentration reduction in encapsulated lactobacillus was smaller (1 $\log_{10}$CFU) (Fig. 2).

Bifidobacterium strain has a completely different behavior. Its gastrointestinal resistance encapsulated is lower than non-encapsulated and completely unable to growth and even survive with bile when encapsulated. Also, it has been a CFU reduction during the encapsulation that made us impossible to get approximately the same starting concentration in bacteria and beads. There is no supernatant growth in any condition. (Fig. 3)

DISCUSSION AND CONCLUSION

Most of the articles related with probiotic beads resistance to gastrointestinal conditions or low pHs show that beads improve the bacterial survival in this conditions but don't emphasize enough how important is the type of bacterial strain they were using.

In our case the behavior of ours bacterial strains when they are exposed to simulated gastrointestinal conditions, even when both of them have probiotic characteristics, was completely different, both encapsulated and non-encapsulated. Resistance of *Bifidobacterium* strain was poor compared with the lactobacilli strain. It is noteworthy that the encapsulated bifidobacteria even has less survival than non-encapsulated, what suggest some negative interaction between polymer and bifidobacteria, lethal for this one.

In the other hand, *Lactobacillus rhamnosus* ATCC 53103™ can survive to each conditions being tested, but in non-encapsulated bacteria case, the fluctuations between the different conditions, especially when get into bile, were higher than in encapsulated bacteria case, which remains stable during the whole process. Also bacteria are transferred to the medium from beads and it is able to growth in it.

In resume, we can conclude that: Lactobacillus do not really need protection for the tested conditions, then no effect of protection is really proved by encapsulation for the tested conditions (Fig.2) and that Bifidobacteria need more protection for bile than simulated gastric conditions but this encapsulation didn't provide protection, even may be detrimental (Fig.3)

Encapsulation of probiotic bacteria with our mixture of microbial polymers can be useful to get an unchanging bacterial concentration during the pass along the gastrointestinal tract, but it will strongly depend on the bacterial strain used, according to our results.

According to these results, we considered that it is needed to focus our future work in an *in vivo* murine model in order to use conditions as similar as possible with human gastrointestinal tract.

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