Controlled release of microencapsulated strains of *Lactobacillus plantarum* in a gastrointestinal tract model.

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INTRODUCTION

Lactobacillus plantarum is a prominent species which has been found to be present in the human and porcine gastrointestinal tract (de Vries et al., 2006). Del Re et al. (2000) showed interesting capacities of adhesion to rectal and jejunal mucosa for 19 strains of *L. plantarum*. This result suggests that bacteria can be found in this part of the intestine. However, dramatic disturbances can occur during critical phases of digestion such as enzymatic secretion, pH modification or slow intestinal transit. Experiments assessing *L. plantarum* viability in *in vitro* conditions simulating the gastrointestinal tract are scarce (Michida et al. 2006). Different strains of *L. plantarum* displayed good viability rates after exposure to hydrochloric acid (pH 2) (Haller et al., 2001). However, this viability is dependent on the extent of the exposure to such conditions and therefore on the transit time. A previous study by Gbassi et al. (2009) showed that *L. plantarum* did not survive at pH 1.8 over 30 min and suggested that microencapsulation improves its tolerance to low pH. This study presents data concerning the impact of pH on the release and viability of *L. plantarum* spp in a gastrointestinal tract model.

MATERIAL AND METHODS

Physiological parameters : The simulated physiological parameters for this gastrointestinal tract model are listed below in the Table 1.

Gastrointestinal tract model					
	STO	DUO	JEJ	IL	CC
Medium	phosphate buffer saline (PBS) 0.05 M (500 ml)				
Transit	2 h	0.25 h	3 h	4 h	1 h
pH values	1.8	4.0	6.0	7.0	7.0
	2.5	5.5	6.5	7.5	7.5
	3.0	6.0	7.0	8.0	8.0
STO : stomach, DUO : duodenum JEJ: jejunum, Il: ileum,CC: caecum					

Table 1: Physiological parameters of the gastro-intestinal tract model

The temperature was maintained at 37°C to represent the human body temperature. Phosphate buffer saline (PBS) was chosen as reference medium. It is the medium commonly used to simulate the intestinal part of the gastrointestinal tract (Michida et al., 2006). The medium was adjusted to various pH values with hydrochloric acid 37% (Riedel-de Haen, Germany) or sodium hydroxide 10 M (VWR, France). The transit time was defined on the basis of the study of Graff et al. (2000). It is

one of the rare studies where the authors measured the gastrointestinal transit times in humans after ingestion of a radiolabelled meal. The pH values of this study were chosen to reflect the changes in pH that might be encountered in an individual (fasted and fed states), as suggested by Vandamme et al. (2002).

Experimental device : The device was a single compartment consisting of a glass beaker surrounded by a thermostated jacket (Figure 1). A pH-meter monitors the pH at each stage of the process. Handlings were carried out in a laminar flow hood.

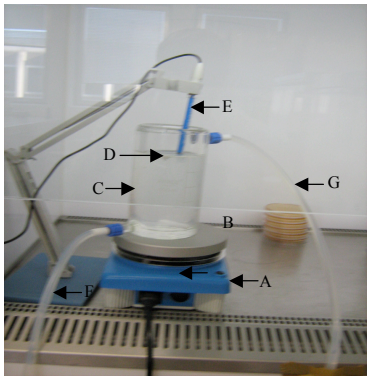

Figure 1. Picture of the experimental device
A: magnetic stirrer, B: magnetic bar,
C: glass beaker with a thermostated jacket,
D: simulated gastrointestinal medium with beads containing *L. plantarum* spp,
E: pH-electrode, F: thermostated water entry,
G: thermostated water exit

Encapsulation of *lactobacillus plantarum* spp : Three strains of *Lactobacillus plantarum* (*L. plantarum* 800, *L. plantarum* CIP A159, *L. plantarum* 299v) were encapsulated in alginate hydrogel beads coated with whey proteins, as described in a previous study (Gbassi et al. 2009).

Release and viability assays in the gastrointestinal tract model : An aliquot of 44 beads of 1.47±0.80 mm was added under stirring (120 rpm) to the medium during 2 h (stomach transit time). Then, the pH was gradually adjusted with sodium hydroxide to the duodenum values for 15 min, jejunum values for 3 h, ileum values for 4 h and caecum values for 1 h. 1 ml of the medium was collected after each transit time for bacterial counting. Serial dilutions were carried out in 0.9% sodium chloride (VWR) and plate counting on MRS agar. After 48 h of incubation, values of bacterial count were expressed as means of three experiments. The bacteria counted in PBS 0.05 M at pH 6.5 after 10 h 15 min were used as controls.

RESULTS AND DISCUSSION

For the controls, the bacterial population in the 44 beads (aliquot) was about 11.30±0.25 for all the strains. Figures 2A, 2B and 2C show the pH-dependent release and viability of microencapsulated strains of *L. plantarum*. Differences between these figures are on the level of pH values applied during the gastrointestinal transit time.

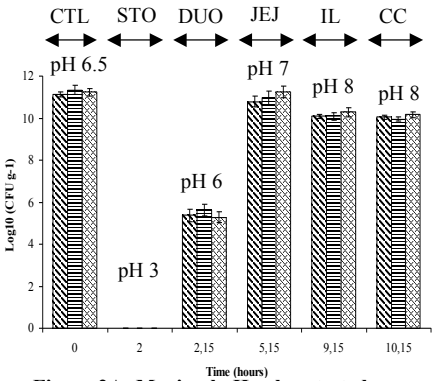

Figure 2A: Maximal pH values tested

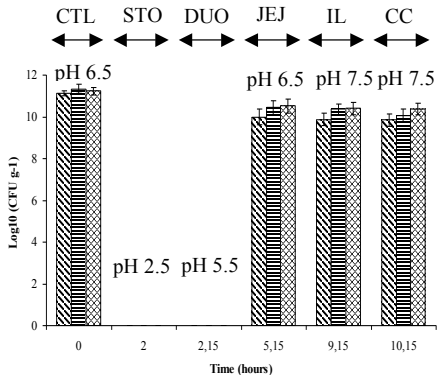

Figure 2B: Intermediate pH values tested

CTL (Controls) STO (Stomach) DUO (Duodenum) JEJ (Jejunum) IL (Ileum) CC (Caecum)

***L. plantarum* 800** ***L. plantarum* CIPA159** ***L. plantarum* 299v**

In figure 2A, no bacteria were counted in the medium after stomach transit time (2 h, pH 3.0). This suggests that the bacteria are either dead or trapped in the beads. The first 15 min of duodenal transit shows an amount of released bacteria into the medium around 5.40±0.20 CFU g^{-1}. This shows that live bacteria remained trapped inside. The three additional hours of jejunal transit achieved 11±0.22 CFU g^{-1} of released bacteria, implying a total release of trapped bacteria. In figure 2B, an amount of 10.30±0.25 CFU g^{-1} was counted in the medium after stomach transit time (2 h, pH 2.5) duodenal transit time (15 min, pH 5.5) and jejunal transit time (3 h, pH 5.5). During the remaining transit time (5 h), the bacterial population remained constant (10±0.20 CFU g^{-1}).

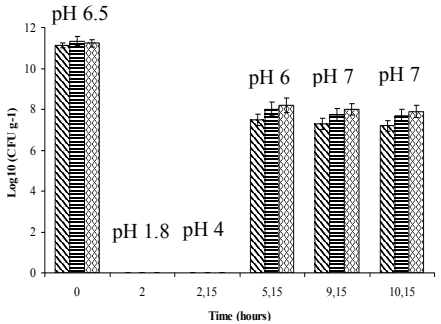

Figure 2C: Minimal pH values tested

In figure 2C, an amount of 7.80±0.40 CFU g^{-1} was counted in the medium after stomach transit time (2 h, pH 1.8) and duodenal transit time (15 min, pH 4.0). A decrease was noted around 3.63 CFU for *L. plantarum* 800, 3.33 CFU for *L. plantarum* CIPA159 and 3.02 CFU for *L. plantarum* 299v. This means that some free bacteria cells were died. During the remaining transit time (5 h), the bacterial population remained constant around 7.55±0.30 CFU g^{-1}.

CONCLUSIONS

The previous study of Gbassi et al. (2009) showed that microencapsulated strains of *L. plantarum* spp survived during 2 h (gastric juice, pH 1.8) after sequential incubation of 30, 60, 90 and 120 min, and during the intestinal transit time (3 h, PBS, pH 6.5). The results of this study showed that the release and viability of bacteria were pH-dependent. Beads sensitivity to pH modifications meets the expected objectives. Studies are being carried out in order to assess the effect of addition of enzymes and bile salts on the release and viability of theses strains.

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