P-026 **Viability of *L. casei* microencapsulated under gastrointestinal conditions**

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INTRODUCTION

Probiotics are live microorganisms which confer health benefits on the host when ingested in sufficient numbers (Araya 2002).They must resist the action of the gastric juice and bile salts as they pass through the stomach and upper intestinal tract (Gismondo 1999).
Microencapsulation has been used to enhance the viability of probiotic bacteria during the processing and storage of food products and in the gastrointestinal tract (Rao 1989).
The aim of this research was to evaluate the resistance of *Lactobacillus casei* CRL 1505 (CERELA-CONICET collection, Tucumán, Argentina) free and encapsulated by the association of ionotropic gelation with Ca^{2+} with complex coacervation using low methoxyl amidated pectin and whey protein, under simulated gastrointestinal conditions

MATERIALS AND METHODS

A pure freeze-dried culture of *Lactobacillus casei* CRL 1505 was activated in MRS broth under aerobic conditions (37 ^{o}C/16 h). The cells were harvested by centrifuging and the pellet obtained was directly suspended in the solution of pectin (2% w/v, pH 4.0). Butter (2% w/v) was added and an emulsion was obtained. The emulsion was atomized in a calcium chloride solution (2% w/v, pH 4.0) and after complete gelation, the microparticles were dispersed in a denatured whey protein solution (90^{o}C/30 min, 8% w/v) and the pH was adjusted to 4.0. The microparticles were frozen and freeze-dried. Free and encapsulated cells were enumerated aerobically by pour plating in sterile MRS agar after 48 h at 37 ^{o}C (Clark 1965).

The survival of free and dried microencapsulated *Lactobacillus casei* after sequential incubation under simulated gastrointestinal were evaluated. Simulated gastric juice was prepared and the pH was adjusted to 1.2 or 2.0 (FAO/WHO 2001). After cooling, pepsin was added to a final concentration of 0.2 mg mL^{-1} (Bermejo 2002). Fresh cell pellets obtained from cultures in the late-log phase (5 mL of MRS broth) or previously rehydrated dried microparticles (sterile water, pH 4.0, 15 min) were added to 2.5 mL of simulated gastric juice and incubated for 0 (control, instantly removed), 1 and 2 h at 37 ^{o}C under agitation.

For enumeration of the microencapsulated *Lactobacillus casei* the bacteria were released from the capsules with sodium citrate (2% at final concentration) at pH 7.0 (0.1M $NaHCO_3$).

After being submitted to the gastric conditions (pH 1.2 or 2.0) for 2 h, 0.1 M $NaHCO_3$ was added to the samples containing the fresh or encapsulated bacteria to adjust the pH to 7.0. A pancreatin solution was added to a final concentration of 0.15 mg mL^{-1}. The samples were stirred for 5 h at 37 ^{o}C and sodium citrate was then added to a final concentration of 2%.

Fresh bacterial pellets or freeze-dried microparticles (0.25 g) previously rehydrated in sterile water (pH 4.0, 15 min), were placed in tubes containing 0.5% or 1% sterile bile salt solution adjusted to pH 7.0, and incubated at 37 ^{o}C for 0 (control, instantly removed) and 5 h under agitation.

Three independent experiments were carried out. For simulated gastrointestinal conditions a 2 X 4 factorial design was used. Bile resistance was evaluated using a 2 x 2 x 2 factorial design. The dates were analyzed by ANOVA and the Tukey test was used to compare the mean results, considering their $\log_{10}$ transformations. The results were considered significantly different at $P < 0.05$.

RESULTS AND DISCUSSION

The free cells showed great sensitivity to the acidic conditions. The initial suspension of free cells showed counts between 8-9 log cfu mL^{-1} and decreased about 4 log cycles after one hour at pH 2.0, the viability being reduced a further two log cycles after 2 h. A non-significant increase of 0.8 log cycles was observed after subsequently raising the of pH to 7 and maintaining it at this value for 5 h. At pH 1.2, when a sample was taken immediately after inoculation, the free cells count decreased about two log cycle as compared to the initial count (8-9 log cfu mL^{-1}), and after one hour the cells were completely and irreversibly damaged.

Table 1: Survival of free and microencapsulated *Lactobacullus casei* (log cfu mL^{-1}) during sequential incubation in simulated gastric juice (SGJ) for 2 h at pH 2.0 or pH 1.2 and simulated intestinal juice (SIJ) for 5 h at pH 7.0 (n = 3).

Sequential incubation		Treatments	
		Free cells	Microencapsulated cells
SIJ pH 1.2	0 min.	8.11 ± 0.39^{aA}	7.82 ± 1.2^{aA}
	60 min.	4.18 ± 0.72^{bB}	7.66 ± 0.75^{aA}
	120 min.	2.02 ± 0.3^{bC}	7.64 ± 0.74^{aA}
SIJ pH 7.0	300 min.	2.82 ± 0.34^{bC}	7.55 ± 0.8^{aA}
SIJ pH 1.2	0 min.	6.56 ± 0.25^{cA}	9.03 ± 0.12^{aA}
	60 min.	$< 1^{bB}$	8.09 ± 0.05^{aB}
	120 min.	$< 1^{bB}$	8.54 ± 0.39^{aAB}
SIJ pH 7.0	300 min.	$< 1^{bB}$	7.17 ± 0.1^{aC}

[a,b,c] For each pH condition (2.0-7.0 or 1.2-7.0), different small letters in the same row differ statistically ($P < 0.05$).
[A,B,C] For each pH condition (2.0-7.0 or 1.2-7.0), different capital letters in the same column differ statistically ($P < 0.05$).

At pH 2.0 the microparticle was able to protect the probiotic bacteria and the cell counts showed no significant differences according to the experimental conditions used. On the other hand, at pH 1.2, the microparticle bacterial count decreased 1.9 log cycles after sequential incubation in simulated gastric and intestinal juice.

At all times the encapsulated bacteria was significantly higher during the sequential incubation in simulated gastric and intestinal juice, as compared to the free cells.

The free and microencapsulated cells showed a slight decrease in their cell counts after 5 h in both bile salt solutions (0.5 and 1.0% w/v). The counts obtained in the 0.5% solution were 8.15 and 9.16 log cfu mL^{-1} at zero time and 7.92 and 8.39 log cfu mL^{-1} after 5 h for free and microencapsulated cells, respectively. When the concentration was increased to 1%, the cell counts obtained were 8.13 and 8.63 log cfu mL^{-1} at zero time and 7.85 and 8.37 log cfu mL^{-1} after 5 h for free and microencapsulated cells, respectively. The standard deviation varied from 0.03 to 0.26.

CONCLUSIONS

The microencapsulation of the *L. casei* CRL 1505 by techniques of ionic gelation and complex coacervation using low methoxyl amidated pectin and whey protein provided significant protection to the probiotic microorganism from harsh acidic conditions, suggesting a potential use for pectin-butter gelled microcapsules coated with whey protein as enteric particles.

However, many important factors concerning the interactions during microparticles production and susceptibility of the bacteria at each step used during production of the particles should be investigated at depth.

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