Microencapsulated formulation of *Lactobacillus casei* protecting probiotic stability in vivo and targeting release

Kristina Mladenovska[1], Katarina Smilkov[2], Tanja Petreska Ivanovska[1], Jasmina Hadzieva[1], Lidija Petrusevska Tozi[1], Zoran Kavrakovski[1], Maja Jurhar Pavlova[3]

[1] Faculty of Pharmacy, University “Ss. Cyril and Methodius”, Skopje, Macedonia
[2] Faculty of Medical Sciences, University “Goce Delčev”, Štip, Macedonia
[3]Faculty of Medicine, University “Ss. Cyril and Methodius”, Skopje, Macedonia

INTRODUCTION

Ingestion of viable probiotic microorganisms results in various health benefits. Maintaining the viability and assuring targeted delivery of probiotics is of great scientific interest, since the production process and the harsh conditions of the gastrointestinal tract often impair the delivery of viable microorganisms in the lower intestine. The first barrier to overcome is the acidic stomach environment.

OBJECTIVES

The objective of this work was to prepare optimal formulation of *L. casei* loaded whey protein-Ca-alginate microparticles with potential for controlled release and colon-targeted delivery of the probiotic.

METHODS

Emulsion technique was applied to aqueous dispersion of alginate and *L. casei* in olive oil, to obtain spherical particles, which were then cross-linked in $CaCl_2$ solution. Microparticles were subsequently coated with native 100% hydrolyzed whey protein isolate, collected, washed and freeze- dried (-50ºC, 0.070 mbar, 24 h).

To deduce the influence of formulation variables, polynomial regression model at 2nd level was used with the experimental matrix of 11 series, and concentration limits of three variables alginate (1 and 4%*m/m*), whey protein (1 and 3%*m/m*) and $CaCl_2$ (1 and 5%*m/m*). The cell load in the initial suspension was ca. 10-11 $\log_{10}$cfu/g.

The viability of microencapsulated *L. casei* was examined in simulated gastric juice (SGJ) (0.08M HCl with 0.2% NaCl and 3g/l pepsin, pH 1.5) for 3 h, simulated intestinal juice (0.05 mol/L KH_2PO_4, pH 6.8) with 1% (w/v) bile salts and 10 g/L pancreatin for additional 3 h and in simulated colon medium (0.1 mol/L KH_2PO_4; pH 7.4) up to 24 h.

The anti-anflammatory properties of the probiotic were assessed by oral administration of the optimal formulation of microparticles once daily (probiotic viability 8,7 $\log_{10}$cfu/g) during 21 days to Wistar rats in which inflammation by intrarectal administration of TNBS in ethanol was induced (10 mg in 0.25 ml 50% ethanol). Clinical score and activity of myeloperoxidase in the colonic tissue were determined after 12 days in comparison with the positive and negative control group and group of rats treated with non-encapsulated probiotic cells.

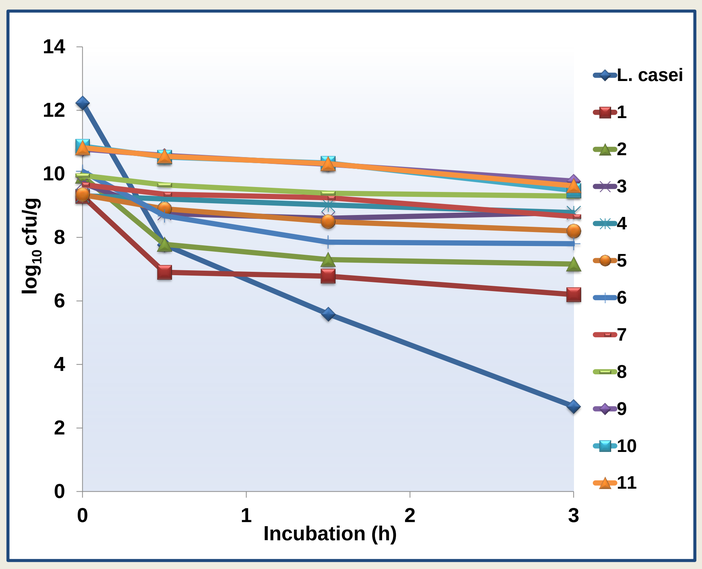

Fig. 1 Viability of the microencapsulated *L. casei* ($\log_{10}$cfu/g) in simulated gastric juice

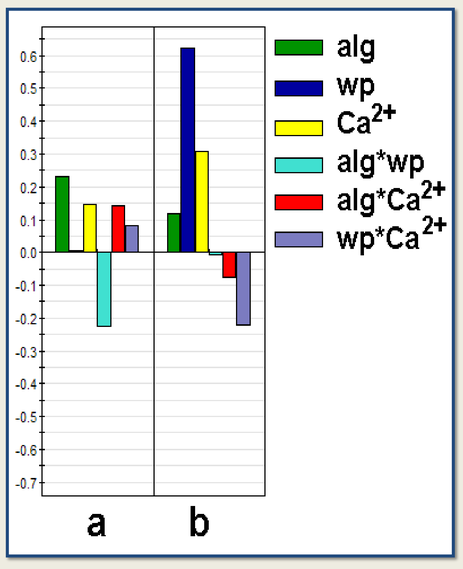

Fig. 2 Effect of the experimental variables on the survival of *L. casei* loaded in whey protein-Ca-alginate microparticles: after freeze-drying (a), after 3 h in SGJ (b).

RESULTS

Eleven series were prepared and negatively charged microparticles were obtained, with size 6.99-9.88 μm after freeze-drying, Ca-content 0.29-0.47 mg/10 mg and viability of the probiotic 9.30-10.87 $\log_{10}$cfu/g. The probiotic load in the microparticles after incubation for 3h in simulated gastric juice containing pepsin was significantly higher (6.20-9.77 $\log_{10}$cfu/g) than the viability of the free cells (2.68 $\log_{10}$cfu/g). (Fig. 1). In addition, the viability of the microencapsulated *L. casei* in simulated colonic condition, pH 7.4, after 24 h was 8.68±0.15 $\log_{10}$ cfu/g.

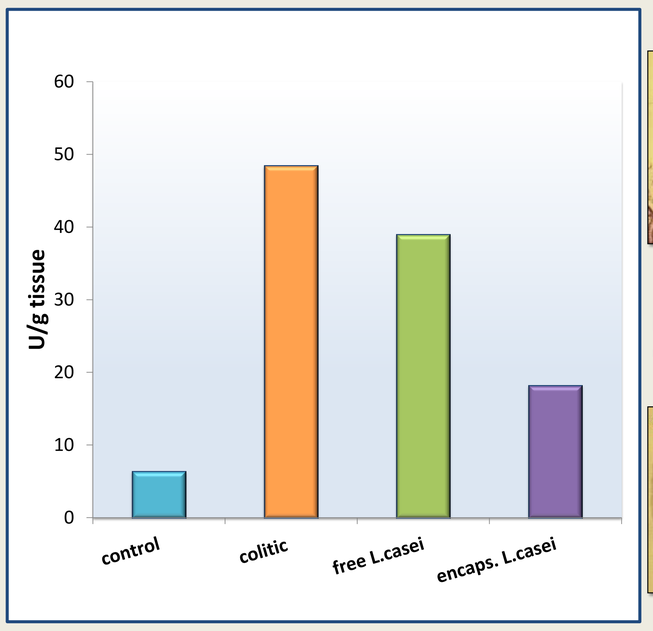

Fig. 3 Myeloperoxidase activity in examined rat groups

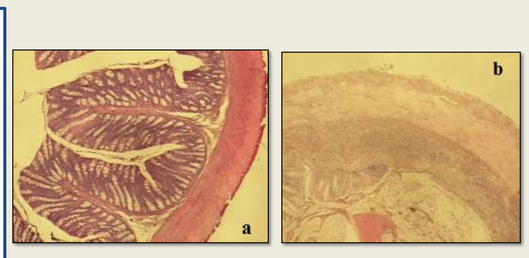

Fig. 4 Transversal section of colonic tissue: control group (a), colitic group (b)

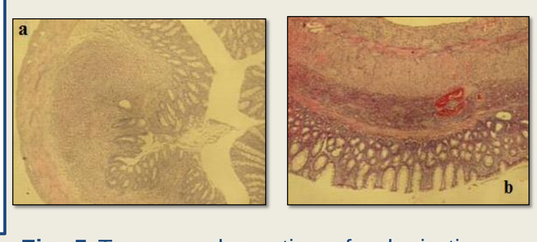

Fig. 5 Transversal section of colonic tissue: group treated with free L.casei (a) and group treated with encapsulated L.casei (b)

The regression coefficients showed that the viability of the probiotic is dominantly (positively) affected by the concentration of whey proteins, followed by the influence of calcium. (Fig. 2). This confirms the protective role of the additional coating of whey proteins, which are not completely degradable in gastric juice and provide the desirable targeted delivery in the large intestine/colon.

The anti-inflammatory properties were confirmed by the significant decrease ($p<0.05$) in the level of myeloperoxidase in Wistar rats, in which TNBS colitis was induced (Fig. 3). These results were confirmed by clinical score and microscopic examination of the transversal section of the extracted colonic tissue (Fig. 4, 5).

CONCLUSIONS

Optimal formulation of *L. casei* loaded whey protein-Ca-alginate microparticles was prepared with physicochemical and biopharmaceutical properties suitable for improving viability of the probiotic cells with anti-inflammatory properties in the GIT and providing their controlled release and colon-targeted delivery.

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