# Anti-inflammatory properties of *L. casei* loaded whey protein-alginate microparticles in animal model of colitis

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## INTRODUCTION

Inflammatory bowel diseases are chronic conditions that affect large population and the drugs used for their treatment have great potential for manifesting adverse effects. Regular administration of probiotics incorporated in pharmaceutical and/or functional food products may significantly prolong, delay or diminish occurrence of these diseases or serve as supplements to conventional drugs. The probiotic *L. casei* has proved its beneficial effects in improving acquired immunity, decreasing colon inflammation, serum cholesterol and increased blood pressure, improving lactose tolerance, controlling irritable bowel syndrome and decreasing risk of colon cancer (Rokka and Rantamaki, 2010). However, as other probiotics, it is easily degraded during production and storage of the final products and, when orally administered, in the aggressive conditions of the GIT. For these reasons, microparticles composed of a probiotic carrier Ca-alginate and coating of whey protein were prepared, where the advantages of the emulsion technique were used to obtain particles with smaller size and high probiotic vability during processing, storage and GI transit and optimal charge for effective colonization in the lower parts of the GIT, especially in inflammatory conditions (Smilkov et al., 2013). The aim of this study was to evaluate the anti-inflammatory properties of *L. casei* loaded in whey protein-alginate microparticles after oral administration to rats in which TNBS-colitis was induced.

## EXPERIMENTAL METHODS

### Materials

Lactobacillus casei-01, freeze-dried probiotic culture was purchased from Chr. Hansen, Denmark. It was activated in MRS broth (Merck KGaA, Darmstadt, Germany) at 37$^{o}$C, 24 h, under aerobic conditions, and the cells were harvested by centrifugation at 1500 g for 10 min and washed twice with sterile 0.1% w/v peptone water (Merck KGaA, Darmstadt, Germany). Alginate (Protanal LF 10/60 LS, fG 35%-45%, IMCD, FMC Biopolymers, Philpadelphia, PA), Tween 80 (Merck KGaA, Darmstadt, Germany) and whey protein isolate (ISO 100, Dynamatize Nutrition, Farmers Branch, TX) were used for preparation of the microparticles.

### Preparation and characterization of microparticles

Emulsion technique was applied to aqueous dispersion of alginate and *L. casei* (10 ml) in olive oil (40 ml) containing 0.2% Tween 80 to obtain spherical particles, which were subsequently cross-linked in $CaCl_2$ solution and coated with whey protein isolate for 1h, isolated, washed and freeze-dried (-50$^{o}$C, 0.07 mbar, 24h, Freeze-Dryer, Labconco, USA). Experimental design was employed to obtain optimal formulation using polynomial regression model at 2$^{nd}$ level with three independent variables: concentrations of alginate, whey protein and $CaCl_2$. Optimal formulation was prepared of 2.5% *w/w* alginate, 3% *w/w* whey protein and 3% *w/w* $CaCl_2$ solutions. Negatively charged, spherical micro-particles were obtained with $d_{50}$ 8.65±1,02μm (Mastersizer Hydro 2000G, Malvern Instruments Ltd., UK), zeta potential -28,04*mV* (Zetasizer Nano ZS, Malvern Instruments Ltd., UK), Ca-content 3.76% (AES-ICP, Varian, CA) and high probiotic viability, 10.55±0.21 $log_{10}$cfu/g after preparation of the microparticles, and 8.68±0.15 $log_{10}$cfu/g in simulated *in vivo* GI conditions (3 h, pH 1.5, 0.08 mmol/L HCl, 0.2% *w/v* pepsin; 3 h, pH 6.8, 0.05 mmol/L $KH_2PO_4$, 1% *w/v* bile salts, 1% *w/v* pancreatin; 18 h, pH 7.4, 0.1 mol/L, $KH_2PO_4$) (Smilkov et al., 2013).

| Parameter | Group of rats | | | |
|---|---|---|---|---|
| | Negative control | Positive control | Non-encapsulated *L. casei* | Encapsulated *L. casei* |
| Colon weight/ total weight (mg/mg) | 0.0067±0.0006 | 0.0076±0.0014 | 0.0072±0.0004 | 0.0070±0.0005 |
| Activity of MPO (U/g) | 8.23±3.32 | 48.43±8.68 | 38.97±6.73 | 18.15±5.10 |
| Total damage score | 0 | 2.6 | 2.0 | 1.8 |

**Table 1. Anti-inflammatory effect of microencapsulated *L. casei***

### Induction of colonic inflammation, experimental design and dosing

To two groups of Wistar rats (n=6/group, 200-260 g, 12-15 weeks) in which TNBS colitis was induced, suspension of free and encapsulated probiotic (optimal formulation), respectively, was administered orally, once daily in amount of 8.7 $\log_{10}$cfu/g vehicle. To the third group, vehicle only was administered (0.25ml milk, positive control), while the forth group of rats (negative control) was treated with 0.25ml PBS, pH 6.8. The colitis was induced in the first three groups after 2 weeks of probiotic and vehicle treatment, which continued for the next 6 days. After 24 hours starvation, in anaesthetized rats, 0.25ml TNBS in 50% ethanol (10 mg/kg) were administered rectally, 8 cm proximally from the anus. Rats were sacrificed after 6 days and the anti-inflammatory effect was evaluated in respect to the clinical activity/total damage score (quantified by loss on weight, consistency of faeces and rectal bleeding), macroscopic and pathohistological changes, colon weight/ body weight ratio and mieloperoxidase (MPO) activity (Peran et al., 2007).

## RESULTS AND DISCUSSION

TNBS model appeared to show high correlation between the pathohistological, immunological and clinical features of the inflammation in IBDs. Comparing to the positive control, the total damage score and colon weight/body weight ratio decreased when *L. casei* was administered, with non-significant difference when free and encapsulated cells were administered (23% and 31% for the total damage score, and 5% and. 8% for the colon weight/body weight ratio, respectively). The activity of MPO was also decreased with the probiotic administration and the lowest value was observed when microparticulated probiotic was administered (Table 1). Macroscopic and histological evaluation confirmed the higher potential of the microencapsulated probiotic to decrease the parameters of inflammation (Figure 1). Visible segments of ulcerations were not observed in the fourth group, while at the histological sections subepithelial polymorph nuclear infiltration was observed with preserved epithelium. Also, in this group, dilated blood vessels in submucosal layer and dilated intestinal glands were observed (Figure 1d).

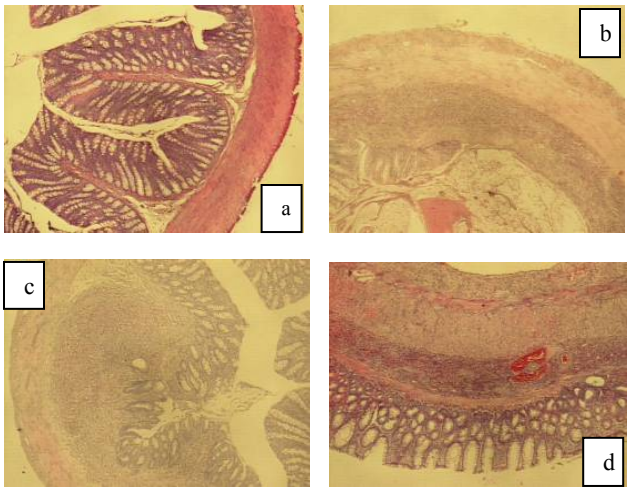


**Figure 1. Optical micrography of rats' colons treated with a: PBS; b: TNBS in ethanol; c: non-encapsulated *L. casei*; b: microencapsulated *L. casei*.**

## CONCLUSION

In conclusion, the microparticluated *L. casei* showed high potential to be used as adjuvant therapy in IBD when incorporated in pharmaceutical dosage form or functional food product.

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