Protein-based micro-beads for improved protection of sensitive ingredients during gastro-intestinal digestion – *in vitro, ex vivo* and *in vivo* evidence.

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INTRODUCTION

There is considerable interest in the development of dietary supplements with physiologically active components that benefit the composition and bio-activity of health-promoting gut microflora. Probiotic bacteria, such as lactic acid bacteria and bifidobacteria are the most widely studied bacteria in the probiotic field and are permanent residence of the intestinal microbiota. However, from a processing point of view, integration of probiotic bacteria into food systems represents a difficult challenge to a food manufacturer. Thus, probiotics should be technologically suitable for integration into different food systems so that they retain viability and efficacy throughout storage and following consumption. The wide use of dairy proteins, in a variety of foods, opens interesting opportunities for milk proteins as cost-effective delivery systems for bioactive compounds such as probiotic bacteria. In a previous study it was shown that entrapment of probiotic bacteria, such as *Lactobacillus rhamnosus* GG (LGG®), in dairy protein-based micro-beads provided excellent storage viability of cells in fruit juice (Brodkorb 2010, Doherty 2011, 2012a). In addition, it was shown that entrapped probiotic bacteria survived in high numbers during simulated *in vitro*/*ex vivo* (Doherty 2012b) and *in vivo* porcine gastric transit.

Coating of microbeads with polysaccharides by electrostatic deposition was also shown to positively influence the stability of probiotic bacteria during gastric transit and could notably delay release in the small intestine (Doherty 2009). However, analytical methods for monitoring effective coating of micro-beads are limited and can be unreliable. In this paper we present a combination of several method used for the characterisation microbeads before and after single/double coating.

MATERIAL AND METHODS

Sample preparation and encapsulation: A milk protein formulation with and without polysaccharides, was rehydrated in distilled water for 16 hours at 4ºC under slight agitation (150rpm). The solution was treated and subsequently stored at 4ºC following neutral pH adjustment using 10 mM HCl (Brodkorb 2010). The bacterial concentrate and protein suspension were in some cases blended, yielding a probiotic population corresponding to the stationary phase concentration (10^9cfu/mL). Monodisperse protein micro-beads were prepared aseptically using an encapsulation device (Inotech Encapsulator®, Dottikon, Switzerland) with a 150µm nozzle size. The beads were agitated gently for a pre-determined time period, subsequently recovered and used immediately for (i) single or double coating or (ii) simulated gastric transit.

Bacterial strain and culture conditions: Some microbeads contained probiotic bacteria, in which case the cell suspension was mixed with the protein solution prior micro-bead production. The probiotic strain Lactobacillus rhamnosus GG (ATCC 53103, Valio Ltd., Finland), was procured from University College Cork, under a restricted materials transfer agreement. Harvested cells were stored as stock solutions in MRS broth (Oxoid Ltd., Hampshire, U.K.) containing 50% (v/v) aqueous glycerol at -20°C. The frozen culture was grown in MRS broth at 37°C under anaerobic conditions; achieved using activated Anaerocult A gas packs (Merck, Darmstadt, Germany). Stationary phase cells destined for encapsulation were propagated from 1% (v/v) inoculums for 19 hours at 37°C. Cells were harvested by centrifugation, washed and re-suspended to obtain a concentrated cell suspension, which was used for the micro-bead production as described above.

Micro-bead Coating: Six different polysaccharide coating materials were kindly donated by Cybercolloids Ltd. (Cork, Ireland) and assays were developed for testing the adsorption efficiency of each coating biopolymer to the protein micro-bead surface. Stock solutions of each biopolymer were autoclaved at 121ºC for 15 minutes. The optimum addition ratio of micro-beads to coating solution was established for each biopolymer solution to facilitate electrostatic deposition of the coating material onto the micro-bead surface (Brodkorb 2010). Coated micro-beads were subsequently recovered from the respective suspension and assayed during ex vivo gastro-intestinal (GI) incubation.

Microscopy: Characterisation of micro-beads and their coating was visually examined under a Leica TCS SP5 confocal scanning laser microscope (CSLM) (Leica Microsystems, Wetzler, Germany). Samples structures were stained using a method involving LIVE/DEAD BacLight® (Invitrogen Ireland) cell viability stain.

In vivo trial: A total of 32 (male) pigs (Large White × Landrace) were weaned at c. 26 days of age. At 14 days post-weaning, (day -7) pigs were tagged, blocked

by mean initial body weight (11.8 ± 1.3 kg) and randomly assigned to one of four treatment groups (n = 8): free-cells, native protein treatment, probiotic micro-beads and coated micro-beads. Samples were administered in apple juice, 4.2 $\log_{10}$ cfu/ml of LGG^{Rif} in respective treatments, which provided a total dose of 1.7 log10 cfu/animal. Two hours post-probiotic administration (day 1), animals from treatment groups A, B and C were sacrificed and the entire GI tract was removed from each carcass immediately after slaughter and digesta (5-10 g) from various GI sections (pyloric gastric region, jejunum, ileum and caecum) were collected and analysed for their probiotic content (LGG^{RIF}).

RESULTS AND DISCUSSION

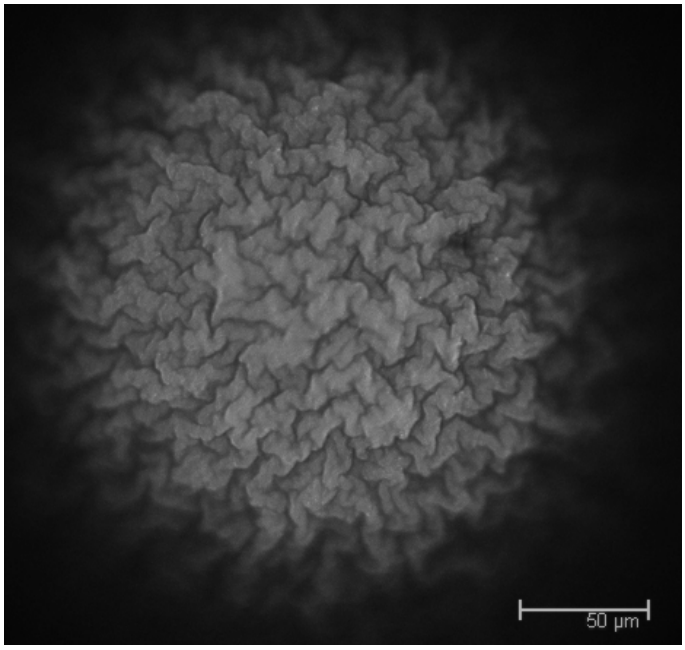

Figure 1: Confocal image of a stained protein microbead.

This study investigated the efficacy of gel micro-beads as encapsulation vehicles to protect probiotic bacteria during porcine gastro-intestinal transit in vivo and deliver viable, functional cells to the small intestine. A rifampicin-resistant derivative of *Lactobacillus rhamnosus* GG (LGG^{Rif}) was incorporated into gel micro-beads prepared from heat-denatured whey proteins. Coated micro-beads were prepared by electrostatic deposition of low methoxylated apple pectin. Content sample of the pyloric stomach, jejunum, ileum and caecum as well as ileum tissue of weaned pigs (n=8 per treatment) were taken two hours after feeding. Analysis included bacterial enumeration (plate counting, flow cytometry), DNA profiling, confocal microscopy and size-exclusion chromatography. Encapsulation in coated and uncoated micro-beads increased survival of LGG^{Rif} by 3 to 4 log10 cycles compared to control treatments (free cells and in presence of native whey proteins). No live LGG^{Rif} was detected in stomach samples of control treatments compared to 4.2 ± 0.2 and 4.4 ± 0.2 log10 cfu/ml for uncoated and coated micro-beads treatments, respectively. Ileal viability increased for coated (7.21±0.59$\log_{10}$ cfu/ml) and uncoated (6.93±0.39$\log_{10}$ cfu/ml) compared to control treatments (3.65±0.76 and4.05±1.13$\log_{10}$ cfu/ml for). Micro-beads maintained their gel structure during gastric transit (pH 1.7±0.3) but disintegrated by proteolytic digestion in the small intestine resulting in a targeted cell release. Coating of micro-beads further delayed release of LGG^{Rif}. Probiotic functionality was maintained during gastro-intestinal transit as viable LGG^{Rif} were detected in the ileum tissue of all treatments.

REFERENCES

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