The use of whey proteins as coating material for improving microbial cells protection : application to probiotic *Lactobacillus plantarum* 299v.

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Introduction

Microencapsulation of probiotics has been investigated for improving their viability in food products and the gastrointestinal tract (Elahi 2008). The biopolymer most commonly used to form microbeads is alginate (Chandramouli 2004). Alginate is used because of its low cost but also for its insolubility in acidic media. Alginate beads can be formed by both extrusion and emulsion methods (Ainsley-Reid 2005; Shima 2006). Poly-cations, such as chitosan or poly-L-lysine, form strong complexes with alginate which are stable in the presence of calcium and reduce the porosity of the gel matrix (Krasaekoopt 2004). Coating alginate beads with poly-cations can improve their chemical and mechanical stability, and thus the effectiveness of encapsulation. On the basis of their amphoteric character, whey proteins can form a complex with surface alginate to obtain a semi-permeable membrane. In this paper, we report a study of the influence of coating alginate beads by whey proteins, and the viability of the microencapsulated probiotic bacteria in the coated beads.

Materials and Methods

Bacterial strain and culture

Lactobacillus plantarum 299v (Lp299v) was purchased from Probis Probiotika (Lund, Sweden) and the strain was routinely grown in MRS broth at 37°C (Biokar diagnostics, Beauvais, France). For encapsulation assays, 100 ml of MRS broth was inoculated with 0.1 ml of an overnight culture and incubated at 37°C for 24 h. Cells were harvested by centrifugation at 4000 rpm for 5 min at 6°C, washed with phosphate buffer saline (Merck, Darmstadt, Germany), and centrifuged again. All the solutions used in our experiments were autoclave-sterilized (120°C, 15 min) except whey proteins and handlings were carried out aseptically in a laminar flow hood (Bioblock scientific, Illkirch, France). Viable cells of Lp299v were determined by serial dilutions in NaCl 0.9%, and plate counting on MRS agar plates following an incubation time of 48 h at 37°C. Values were expressed as means of duplicate determinations.

Technique of microencapsulation used

After washing, the cultures were mixed with 15 ml of sterile 2% (w/v) sodium alginate solution (Fluka AG, Buchs, Switzerland). The cell suspension was extruded through a 27.5 G bevelled needle (Teruma) into a sterile solution of 0.05 M $CaCl_2$. The beads were allowed to stand for 30 min for gelification, and then rinsed.

Coating procedures
Whey proteins were selected for their resistance in conditions simulating the gastric juice. They were analyzed by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). Whey proteins were dissolved in deionized water (2%, w/v), filter sterilized and aliquoted in 25 ml vials: vial A, set at pH 7.0; vial B, set at pH 1.8; vial C, set at pH 1.8 and containing pepsin (0.3%, w/v). The three vials were incubated at 37°C for 4 h under agitation (125 rpm). 50 µl of each vial were mixed with 50 µl of a sample buffer consisting of 2.5 ml of pure glycerol (Fluka AG, Buchs, Switzerland), 0.5 ml of 2-mercaptoethanol (Bio-Rad, Hercules, USA), 2 ml of sodium dodecyl sulphate (10%, w/v) (Bio-Rad, Hercules, USA), 1.25 ml of 0.5 M Tris HCl pH 6.8 (Usb, Swampscott, USA), 0.2 ml of bromophenol blue (0.5%, w/v) (Ems, Washington, USA) and 3.55 ml of deionized water for a total volume of 10 ml. In a second time, 10 µl of each previous vial were mixed with 40 µl of deionized water and 50 µl of sample buffer to obtain vials A', B' and C'.
The beads of alginate were coated by immersion in 2% whey proteins solution and vortexed. The uncoated beads were used as controls. Coated and uncoated beads were freeze-dried (alpha 1-4 LSC, Fischer Bioblock, Illkirch, France).

Analysis of number of entrapped cells, size and beads morphology
Freshly prepared beads (0.2 g) were dispersed in 10 ml of 0.05 M sterile phosphate buffer (pH 6.8) by gentle shaking at 37°C. After beads dissolution, Lp299v was enumerated on MRS agar after incubation at 37°C for 48 h. Size and shape were determined using a scanning electron microscope (SEM) (Philips XL 20, Oregon, USA). After gold metallization under a gentle nitrogen flow during 5 min in a vacuum chamber, the microstructure of beads was analyzed on the surface and after a cross-section.

Survival of microencapsulated cells in simulated gastric juice
Freshly prepared beads (0.2 g) were placed into a tube containing 10 ml of simulated gastric juice (SGJ) (0.9% NaCl, pH 1.8, 0.3% pepsin from porcine stomach mucosa) and incubated at 37°C for 30, 60, 90, and 120 min. The beads were then removed and placed in 10 ml of phosphate buffer. 0.1 ml aliquot of dissolved beads was removed and viable cells were enumerated using methods described in section 2.1.

Results and Discussion

Encapsulation and coating
The results of SDS-PAGE (Fig. 1) showed that whey proteins are stable in acidic media. Low pH had no effect on the composition and structure of whey proteins. Analysis of figure 1 revealed that whey proteins are composed of three proteins, the first below 75 KDa, the second over 15 KDa and the last between 10 and 15 KDa. These proteins have been identified respectively as bovine serum albumin, β-lactoglobulin and α-lactalbumin, as described elsewhere (Kitabatake et al., 1998). No difference was observed between the proteins A (pH 7.0) and B (pH 1.8), and A' (pH 7.0) and B' (pH 1.8) . This demonstrates that whey proteins remain intact after 4 h of exposure to pH 1.8. In the presence of pepsin, bovine serum albumin and α-lactalbumin are degraded, but β-lactoglobulin remains intact. This finding is consistent with that of Li et al. (2004) who showed that β-lactoglobulin, the major protein of whey, is stable to *in vitro* digestion.

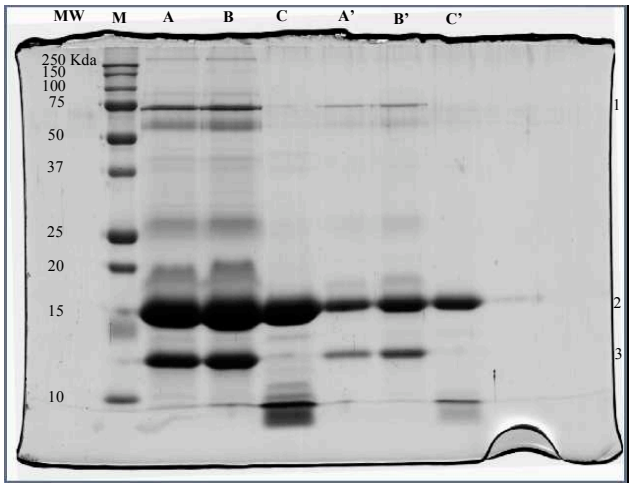

Fig. 1. Electrophoresis profile of whey proteins in SGJ (MW: molecular weight; M: Marker; A-A': whey proteins at pH 7.0 as control; B-B': whey proteins at pH 1.8; C-C': whey proteins at pH 1.8 plus pepsin. A', B' and C' are the dilutions of A, B and C)

Number of entrapped cells, size and beads morphology

Initial cell numbers in the alginate beads is about 10^{10} cfu g^{-1} with the microencapsulation technique described. Size and shape of beads were observed by using a SEM. As shown in Fig. 2A, oval and spherical beads were observed, the average diameter of the beads was 1.47±0.80 mm. SEM photographs of cross-sections of alginate beads coated with whey proteins revealed that encapsulated bacteria were distributed at random and equally throughout the alginate network (Fig. 2B).

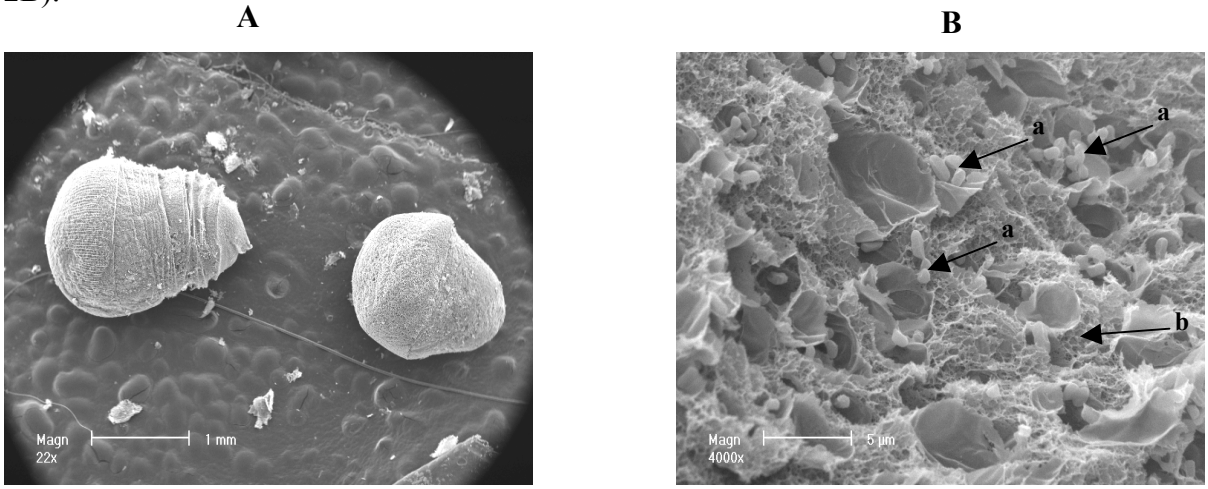

Fig. 2. A: SEM photograph of alginate beads coated with whey proteins, B: Internal structure of beads (a) bacteria (b) alginate gel matrix

Survival of microencapsulated cells in simulated gastric juice

Bacterial cells in coated beads after exposure to SGJ with and without pepsin were respectively 10^6 and 10^7 cfu g^{-1} after 120 min (Fig. 3). At the same period, no survival was observed with the uncoated beads. Coating with whey proteins provided an even better protection compared to uncoated beads. When SGJ contains pepsin, the survival of Lp299v is reduced by cycle of 1 log cfu g^{-1}.

In spite of the presence of pepsin, whey proteins remain effective in protecting Lp299v. The choice of pH 1.8 reflects the acidity of human gastric juice but also the sensitivity of free Lp299v to this pH, in order to assess the benefit of coating on the survival of probiotic Lp299v.

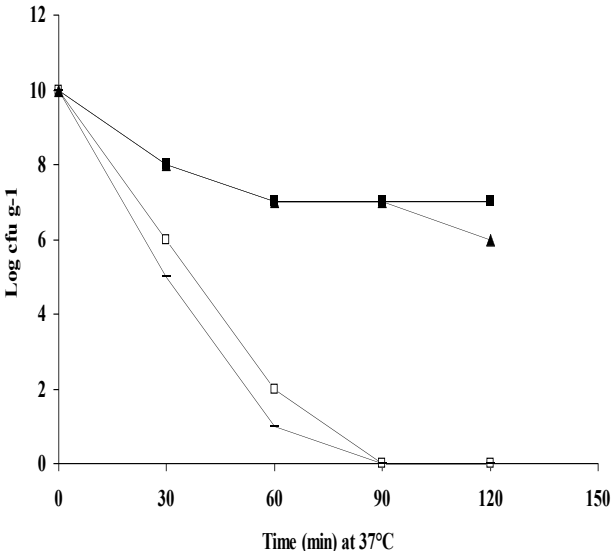

Fig. 3. Viability of immobilized cells with and without coating after exposure to gastric juice (■ Coated beads at pH 1.8 ▲ Coated beads at pH 1.8 plus pepsin □ Uncoated beads at pH 1.8 - Uncoated beads at pH 1.8 plus pepsin)

Conclusion

This preliminary work showed that whey proteins can be used as a convenient material for improving Lp299v protection. Coating the alginate beads with whey proteins provided an even better protection. Due to its low cost, biocompatibility and resistance in gastric juice, whey proteins can serve as vehicles for sensitive ingredients through the stomach.

References

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