Simulated gastric and intestinal fluid survival of *Bifidobacterium longum* Bb-46 encapsulated in different interpolymer complexes

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1. Introduction

Probiotics must survive transit through the gastrointestinal tract and reach the colon in large quantities to facilitate colonization in the host (Alander et al., 1999; Hsiao et al., 2004; Mainville et al., 2005). In the large intestine ingested bacteria compete for nutrients and adherence sites on the intestinal epithelium with already established microbiota comprising several hundreds of other bacterial species (Alander et al., 1999). The sensitivity of bifidobacteria to acidic environments presents a challenge for application of these microorganisms in different industries (Hansen et al., 2002).
Protection of bifidobacteria, specifically by use of microencapsulation, has been attempted by various researchers (Cui et al., 2000; Lee and Heo., 2000; Sultana et al., 2000; Sun and Griffiths, 2000; Guérin et al., 2003; Lian et al., 2003; Capela et al., 2006). The different encapsulation developed aimed at ensuring greater survival of probiotic bacteria under gastric conditions. Most of these methods indicated the potential for application of encapsulation of probiotic bacteria in food and pharmaceuticals as encapsulated bacteria survived better than their non-encapsulated counterparts. However, more research still needs to be done on the methods for optimum protection of encapsulated bacteria to be obtained. The methods developed use solvents which may negatively affect survival of encapsulated cells as they are sensitive to solvents and there are environmental concerns about use of solvents. Solvents should be avoided in order to improve chances of survival of encapsulated probiotic cultures. Encapsulation of probiotics in $scCO_2$ was reported for the first time by Moolman et al. (2006, in press). The aim of this study was to investigate the survival of interpolymer complex encapsulated *Bifidobacterium longum* Bb46 in simulated gastric and intestinal fluids, and to investigate effects of different modifications of the polymers on bacterial survival.

2. Materials and methods

2.1 Encapsulation of bacteria

Freeze-dried *B. longum* Bb-46 (Chr. Hansen) was warmed to room temperature. 2 g of the bacteria was ground using a coffee grinder (Model CG100, Kenwood) to a powder passing through a 150 µm sieve. PVP (Kollidon 12PF, mass-average molar mass 2 000 – 3 000 g/mol, BASF) pre-dried for 5 hours at 80 ºC and 60 mbar in a vacuum oven (Model VO65, Vismara) and stored in a desiccator and PVAc-CA (Vinnapas C305, mass-average molar mass 45 000 g/mol, Wacker) were added to the bacteria, together with any additives (e.g. glyceryl monostearate – Croda Chemicals). The blend was then ground and mixed for 1 minute. The powder blend was immediately transferred to the pre-heated 1 L reaction chamber wiped dry with 70% ethanol. The chamber was then sealed and flushed and pressurized with sterile filtered CO_2 (99.995% purity, Air Products) up to a pressure of 300 bar, with the temperature controlled at 40 ºC. The material was left to equilibrate for 2 hours with intermittent stirring, after which the plasticized product was sprayed through a 500 µm capillary with length 50 mm, into a 10 L expansion chamber that was pressure-controlled at 15 bar (gauge).

2.2 Preparation of simulated gastric and intestinal fluids

Simulated gastric juice (SGJ) was prepared according to Lian *et al*. (2003) while simulated intestinal fluid (SIF) was prepared according to US Pharmacopeia (2005).

2.3 Survival of bacteria in SGF

1 g of bacteria was added to 9 mℓ SGF (37 ^{o}C, pH 2.0) in a test tube and vortexed for 30 s for complete dispersion. Samples were taken immediately after vortexing to determine viability of bacteria. The test tubes were then incubated at 37 ^{o}C in a shaker incubator (50 rpm) for 2 h. 1 mℓ aliquots were removed from the tube at times 0.5, 1 and 2 h for enumeration of bifidobacteria. Test tubes with encapsulated bacteria were not vortexed during sampling so as not to interfere with release of bacteria from the interpolymer matrix.

2.4 Survival of bacteria in SIF

1 mℓ of non-encapsulated and encapsulated bacteria from the SGF survival test were each suspended in 9 mℓ of SIF (37 ^{o}C, pH 6.8) in a separate test tube and vortexed for 30 s for distribution of the sample. Excess SGF from the tube containing encapsulated material was discarded. The remaining solids were resuspended in 9 mℓ of SIF. 1 mℓ samples were taken from the three tubes immediately after suspension in SIF, for enumeration of bifidobacteria. The tubes were then incubated at 37 ^{o}C in a shaker incubator at 50 rpm for 6 h. Samples were taken after 2, 4, and 6 h for bifidobacteria enumeration.

2.5 Enumeration of *Bifidobacteria*

Serial dilutions of the 1 mℓ samples taken were prepared in sterile Ringer's solution (pH 7). 100 µℓ of appropriate dilutions were plated out in triplicate on MRS (Merck, Pty.Ltd) agar plates supplemented with 0.05 % cysteine hydrochloride. The plates were incubated at 37°C for 72 h in anaerobic jars with Anaerocult A gaspaks (Merck, Pty.Ltd) and Anaerocult C test strips for indication of anaerobic conditions inside the jar. Colonies grown were counted and from these numbers of viable bacteria were calculated (cfu/g).

3. Results and Discussion

Encapsulation of *Bifidobacterium longum* Bb-46 in the basic system improved survival of the bacteria by 1.42 log cfu/g (Fig.1). However, incorporation of PEO-PPO-PEO (Fig. 1) and replacing PVP with PCL (Fig. 2) resulted in decreased survival of bacteria ending up with counts of released live bacteria form the interpolymer complex less than the control counts. Protection efficiency of the interpolymer matrix was improved by incorporation of 8% GMS in the interpolymer complex resulting in improved survival of encapsulated bacteria by 2.2 log cfu/g when compared with controls (Fig. 3). Increasing the GMS loading in the interpolymer complex did not improve protection efficiency as was anticipated (Fig. 4). Gelatine capsules only delayed contact between the detrimental gastric fluid and bifidobacteria but did not improve the survival at the end of exposure period (Fig. 5). Beeswax:PVP improved survival of bacteria by 1 log (Fig. 6). All the interpolymer complex formulation improved survival of bacteria when compared to controls indicating the potential of the interpolymer complex for protection of sensitive bacteria in the journey through the gastrointestinal tract.

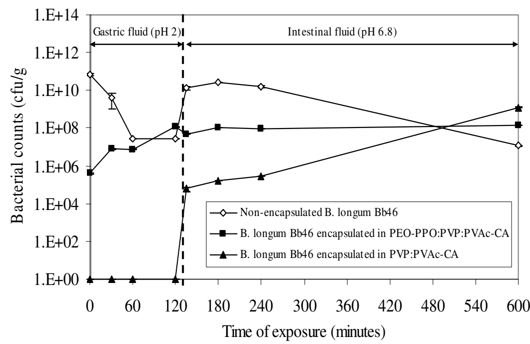

Fig. 1: Survival of *B. longum* Bb-46 encapsulated in PVP: VA-CA and PEO-PPO:PVP:VA-CA after exposure to SGF and SIF

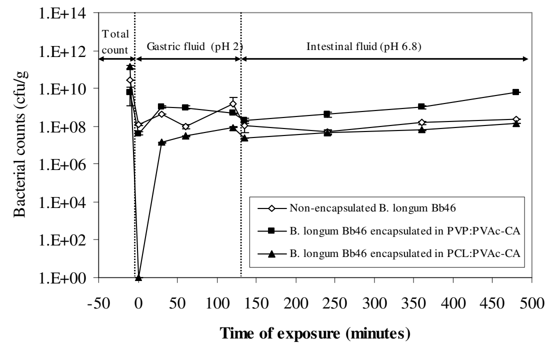

Fig. 2: Viability of *B. longum* Bb-46 cells encapsulated in PCL:VA-CA and PVP:VA-CA after exposure to SGF and SIF

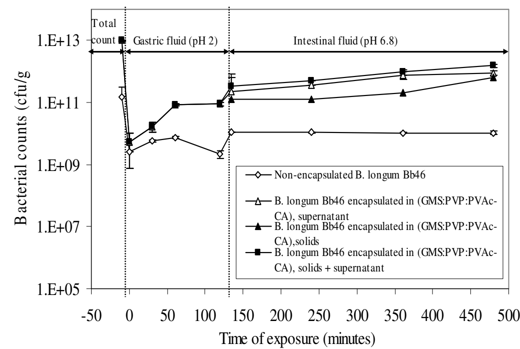

Fig. 3: Effect of GMS incorporation into the interpolymer complex on survival of *B. longum* Bb-46 after exposure to SGF and SIF

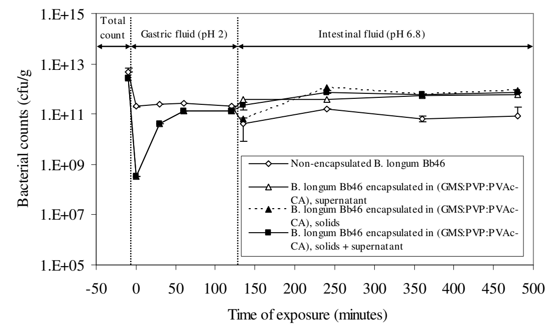

Fig. 4: Effect of higher loading of GMS on survival of *B. longum* Bb-46 after exposure to SGF and SIF

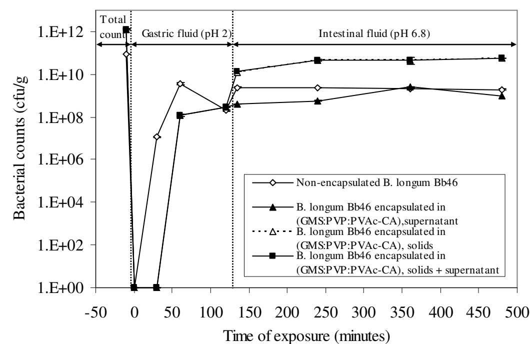

Fig. 5: Effect of enclosure of GMS:PVP:VA-CA encapsulated *B. longum* Bb-46 into gelatine capsules on survival after exposure to SGF and SIF

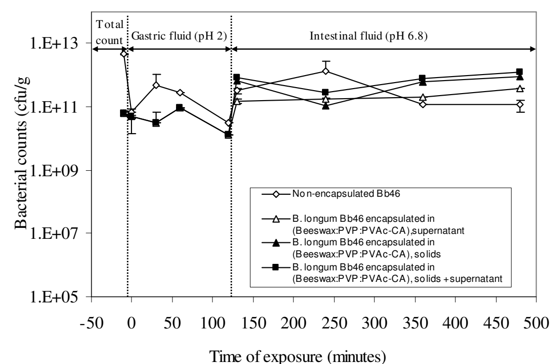

Fig. 6: Effect of beeswax incorporation into the interpolymer complex on survival of *B. longum* Bb-46 after exposure to SGF and SIF

5. Conclusions

PVP: CA-CA interpolymer complex protected *B. longum* Bb-46 from SGF and then released cells in SIF for colonization. Presence of 8% GMS in the interpolymer matrix improved protection efficiency of PVP:VA-CA. Use of PCL, PEO-PPO-PEO and beeswax did not enhance protection of encapsulated bacteria by the interpolymer complex.

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