IMMOBILIZATION OF MICROORGANISMS IN FIBERS

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Abstract

Probiotics have been defined as live microbes which transit the gastro-intestinal tract and in doing so benefit the health of the consumer. They have to be able to resist to gastric acidity and bile salts in the small intestine. A method for encapsulation and stabilization of probiotic yeast (Saccharomyces boulardii) or bacterial strains (Lactobacillus and Bifidobacterium spp.) in polymeric or biopolymeric fibers has been developed. Centrifugal methods using a rotating bell have been employed to produce a mixture of nano- and microfibers. Water solutions of polyvinylalcohol and gelatine with prebiotic fiber inulin and suspended microbial preparations have been used produce the fibers. The centrifugal spinning is simple, cheap and eco-friendly process. The process can be carried out at laboratory temperature without any heating. The survivability of probiotics in the fibrous preparations under in vitro conditions was conducted in a bile salts solution and a simulated gastric juice, followed by incubation.

Keywords: probiotic bakteria; Saccharomyces boulardii; immobilization; micrpfibers; PVA; gelatine

1. INTRODUCTION

Probiotics have been defined as live microbes which transit the gastro-intestinal tract and in doing so benefit the health of the consumer. They have to be able to resist to gastric acidity and bile salts in the small intestine. A method for encapsulation and stabilization of probiotic yeast (*Saccharomyces boulardii*) or bacterial strains (*Lactobacillus* and *Bifidobacterium* spp.) in polymeric or biopolymeric fibers has been developed. Centrifugal methods using a rotating bell have been employed to produce a mixture of nano- and microfibers. Water solutions of polyvinylalcohol and gelatine with prebiotic fiber inulin and suspended microbial preparations have been used produce the fibers. The centrifugal spinning is simple, cheap and eco-friendly process. The process can be carried out at laboratory temperature without any heating. The survivability of probiotics in the fibrous preparations under in vitro conditions was conducted in a bile salts solution and a simulated gastric juice, followed by incubation.

2. MATERIALS AND METHODS

2.1 Material

Spray dried microbial preparation BA (1.109 CFU.g-1) (Milcom, Czech republic), containing L*actobacillus acidophillus* and *Bifidobacterium bifidum* probiotic bacterial strains and dried milk. Freeze dried *Saccharomyces boulardii* yeast strain was purchsed from the Czech Collection of Microorganisms (CCM). Preparation of the polymeric microfibers with incorporated microbial cells. A polyamide bell with an upper diametr 12 cm, a bottom diametr 10 cm and a height 8 cm (see Fig. 1) was used for the centrifugal microfiber production. The bell rotated at a constant velocity in the range 2000 - 8000 rpm using an electromotor. The microbial suspensions in the polymeric solutions with inulin were pumped to the bell bottom by a tube with a constant flow rate in the range 1 - 5 ml.min^{-1}. The microfibers formed at the edge of the rotating bell were collected on a scaffold made from a stainless steel (see Fig. 2).

2.2 Composition of the spun suspensions and obtained fibers:

2.2.1 Gelatine - Saccharomyces boulardii

Composition of the spun suspension: gelatine (gel strength 300, Type A, Sigma) 26 -30 % w/w, acetic acid 10 - 40 % w/w, yeast biomass DM 17,3 % w/w.

Composition of the fibers DM: gelatine 59,8 % w/w; yeast biomass DM 40,2 % w/w.

2.2.2 Enzymatically crosslinked gelatine - inulin - *Lactobacillus acidophillus* and *Bifidobacterium bifidum*

Composition of the spun suspension: gelatine 24 - 30 % w/w, acetic acid 10 - 40 % w/w, inulin (Frutafit HD, Brenntag) 1,7 - 2,2 % w/w, microbial preparation BA 1,7 - 2,2 % w/w, transglutaminase (Activa WM, Ajinomoto) 0,75 - 1,1 % w/w.

Composition of the fibers DM: 84,8 % w/w; inulin 6,1 % w/w; microbial preparation BA 6,1 % w/w; transglutaminase 3 % w/w.

2.2.3 Physically crosslinked polyvinyl alcohol (PVA) - inulin - *Lactobacillus acidophillus* and *Bifidobacterium bifidum*

Composition of the spun suspension: PVA (Sloviol R, Fichema) 10 % w/w; inulin 3,9 % w/w; microbial preparation BA 3,9 % w/w; pH 4,3.

Composition of the fibers DM: PVA 56,1 % w/w; inulin 21,95 % w/w; microbial preparation BA 21,95 % w/w.

PVA in the microfiber was crosslinked by the freeze-thawing process, as described previously [1, 2].

2.2.4 Determination of resistance of the encapsulated probiotics to bile salts and simulated gastric acidity

The standard method was used for the enumeration of vital bacteria on MRS agar (Oxoid Ltd.) plates in the fiber products and control samples after 1 hour treatment at pH 2, adjusted with HCl. Resistance to bile acids was tested by incubation in the MRS agar medium supplemented with 0,5 % w/v standardised ox-biles extract (Oxoid Ltd.) in an anaerobic jar for 48 h at 37ºC.

Fig. 1 Polyamide bell for the centrifugal microfiber production

Fig.2 Collector of microfibers

3. RESULTS

3.1 Preparation of the polymeric microfibers with incorporated microbial cells

Production rates of the centrifugal spinning system was up to 32 g of the PVA microfibers DM and up to 20 g of the gelatine microfibers DM per hour. The obtained microfiber mats have cotton wool like appearance (Fig. 3). Diametrs of the produced fibers were in the range of 1 - 10 μm (Fig. 4). Each of the fibers depicted by the optical microscopy was composed from smaller nanofibers and microfibers twisted into bundles. The microbial cells were incorporated inside the fibers, as shown in Fig. 5.

3.2 Determination of resistance of the encapsulated probiotics to bile salts and simulated gastric acidity

Enumeration of the vital yeast cells in the gelatine fibers has not been finished yet. Taking into consideration high concentrations of acetic acid necessary for a satisfactory process of centrifugal spinning of gelatine solutions, we have observed a steep decrease of bacterial vitality by several orders (results not shown). There was no decline of the original bacterial viability because of the encapsulation process using PVA polymer. However, encapsulation of the probiotic bacteria in the PVA microfibers didn't influence their resistance to simulated gastric acidity and bile acids in comparison with the control sample significantly. Declines by 2 orders were observed with both the samples.

Fig. 3 PVA microfiber mat with the encapsulated probiotic bacteria

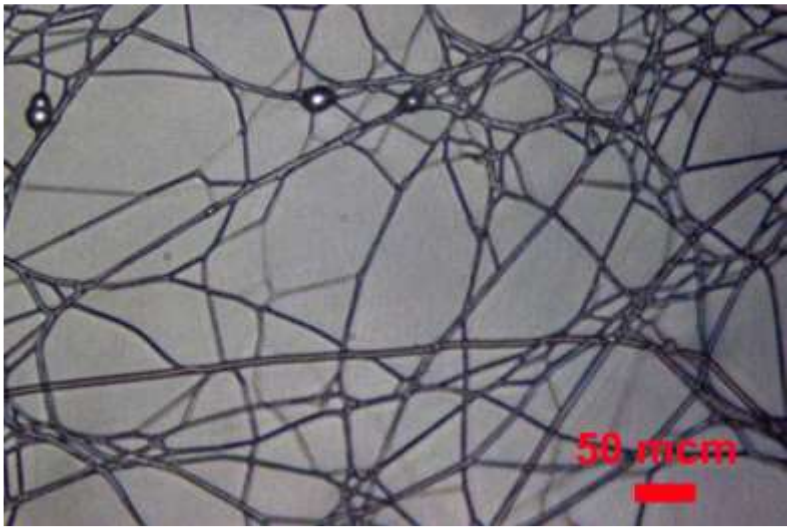

Fig. 4 Detail of PVA microfiber mat with the encapsulated probiotic bakteria depicted by optical microscopy

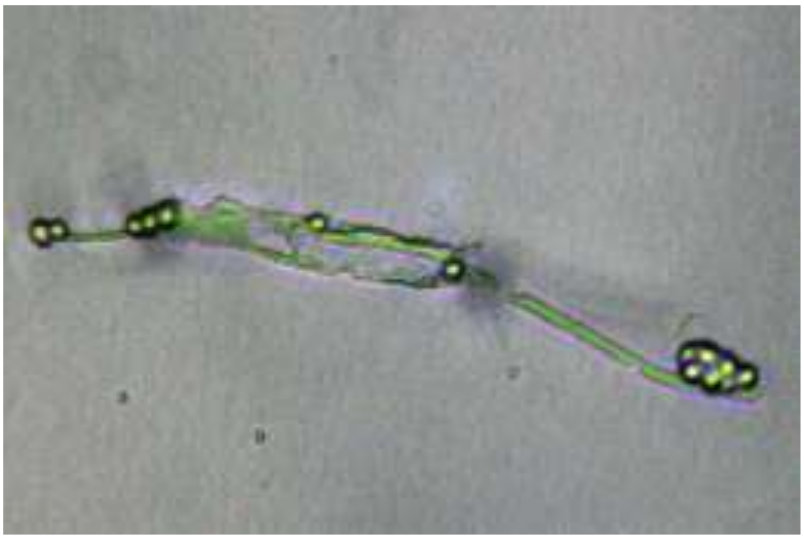

Fig. 5 Imaging of *Saccharomyces boulardii* yeast cells in a gelatine microfiber by optical microscopy

4. DISCUSSION

Encapsulation of probiotic bacteria in polymeric microfibers and nanofibers by electrospinning have already been described in several publications [3-5] and it was also patented [6]. Encapsulation of probiotic microorganisms in micofibers by the centrifugal spinning has several advantages in comparison with the electrospinning process. It is cheaper, simple, more productive, easy to up-scale,

and more gentle to the microorganisms. Moreover, it doesn‘t require the high voltage electric fields to transform polymer solutions into nanofibres. In the case of spinning of biopolymeric solutions, it can be often realized with Loir concentrations of organic acids.

CONCLUSIONS

High concentrations of acetic acid which are necessary for a satisfactory process of the centrifugal spinning of gelatine solutions resulted in a steep decrease of bacterial vitality by several orders. The technology is

currently being optimized with the aim to decrease the acetic acid concentration in gelatine solutions and to minimize the contact time of the probiotic microorganisms with the acidic solution. Encapsulation of the probiotic bacteria in the PVA microfibers didn't decrease their viability but had no significant effect on the resistance of the encapsulated probiotics to simulated gastric acidity and bile salts. One of the possible explanation for inability of the PVA fibrous encapsulant to protect microorganisms against the negative environmental factors in the GI tract can be a limited effectivity of the physical method to crosslink the polymer. At present, long term viability tests of the encapsulated probiotic bacteria and yeasts in dry form and in several food products are in progress to confirm the beneficial supporting effects of the biopolymeric fiber matrix on microbial survival.

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