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Enhanced viability of probiotics (Pediococcus *pentosaceus* Li05) by encapsulation in microgels doped with inorganic nanoparticles

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ABSTRACT

The major reduction in probiotic viability that occurs during food storage and gastrointestinal transit limits their potential health benefits. Microencapsulation is one of the most effective methods to protect probiotics from various harsh conditions. In this study, a model probiotic (*Pediococcus pentosaceus* Li05) was encapsulated in an alginate-gelatin microgels in the absence and presence of magnesium oxide (MgO) nanoparticles (NPs). The morphology and surface properties of the encapsulation systems were characterized by transmission electron microscopy (TEM) and atomic force microscopy (AFM), which showed that both the probiotics and NPs were successfully incorporated into spherical microgels. The viability of the probiotic was evaluated after exposure to different conditions: long-term storage in an aerobic environment; heat treatment; and gastrointestinal transit. Encapsulation of the probiotics significantly enhanced their viability under these different conditions. Probiotics encapsulated in MgO-loaded microgels were more stable than free bacterial cells or those encapsulated in microgels alone: less than 2 $\log_{10}$ CFU reduction after 40 min incubation in gastric fluids versus 5 $\log_{10}$ CFU reduction in the first 10 min for free cells. The SEM images indicated that the NPs may lead to enhanced probiotic viability by filling pores inside the microgels, which may have inhibited the ability of oxygen and hydrogen ions to access the probiotics. Moreover, the MgO NPs neutralized the hydrogen ions in the gastric fluids, thereby reducing acid-induced degradation of the probiotics. These results demonstrate that MgO-loaded microgels may be a promising encapsulation and delivery system for improving the efficacy of orally administered probiotics by protecting them from the harsh conditions during storage and in the gastrointestinal tract.

1. Introduction

The human gut microbiota plays an essential role in human health and disease, aiding in food digestion as well as the establishment and maintenance of the immune system (Artis, 2008; Cho & Blaser, 2012; Lynch & Pedersen, 2016). Probiotics have been used as treatments for various diseases due to their ability to promote a healthier gut microbiota (Bron et al., 2017). Consequently, a considerable amount of research is being carried out to identify bacterial species with potential health-promoting functions. However, most probiotics are highly susceptible to the harsh conditions present in many foods and within the human gastrointestinal tract (GIT). Despite being widely used in commercial products, the viability of probiotics is usually not in accordance with the claims made on food packaging (Drago, Rodighiero, Celeste, Rovetto, & De Vecchi, 2010). This is because probiotics are sensitive to many stress factors that significantly reduce their viability and bioactivity. For instance, probiotics lose viability during food storage or processing due to high oxygen levels and heat treatments (Sanders & Marco, 2010). Besides, probiotics also lose viability as they move through the GIT due to the presence of gastric acids, enzymes, and bile salts (Cook, Tzortzis, Charalampopoulos, & Khutoryanskiy, 2012).

Encapsulation of probiotics into microcapsules can be used to enhance the viability and stability of probiotics in foods and the GIT (Cook et al., 2012; Solanki et al., 2013). Encapsulation of probiotics (bifidobacteria) in microgels consisting of an alginate core and a chitosan coating improved their viability in gastrointestinal fluids (Yeung, Ucok, Tiani, McClements, & Sela, 2016). Encapsulation of another probiotic (lactobacillus) in microgels consisting of pectin was also shown to greatly improve its viability within the GIT (Li et al., 2016). Previously, we prepared microgels from a mixture of alginate and

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gelatin and showed that they could enhance the viability and delivery of *Lactobacillus salivarius* Li01 to the gut (Yao et al., 2017). However, these microgels were porous, with a pore size around 17 nm, and so small ions and molecules in the surrounding solutions (such as hydrogen ions, bile salts, or digestive enzymes) could easily diffuse into them, which may lead to degradation of the encapsulated probiotics (Huq et al., 2017; Zhang et al., 2016). This problem may be overcome by the addition of inorganic NPs to the microgels to fill in the pores (Huq et al., 2017). Moreover, certain types of inorganic NPs, such as Mg (OH) $_2$, can be used as buffers to neutralize hydrogen ions and maintain a neutral pH inside the microgels even when they are suspended in gastric fluids (Zhang, Zhang, Sun, Park, & McClements, 2016). These types of NPs may therefore be able to improve the viability of encapsulated probiotics by restricting diffusion and by controlling the internal pH.

In this study, the efficacy of alginate-gelatin microgels loaded with MgO NPs in protecting a model probiotic was investigated. *Pediococcus pentosaceus* Li05 strain (CGMCC 7049) isolated from the fecal samples of healthy volunteers was used for this purpose. Oral administration of *P. pentosaceus* Li05 has been shown to decrease acute liver injury in rats (Lv, Hu, et al., 2014). However, its low viability in harsh conditions prevents it from the widespread application in functional foods and pharmaceuticals. The results from this study will facilitate future applications of P. *pentosaceus* Li05 as a functional food or pharmaceutical.

2. Materials and methods

2.1. Materials

DeMann, Regosa, Sharpe (MRS) broth and *Lactobacilli* MRS agar were purchased from Oxoid (Oxoid, Basingstoke, Hampshire, UK). Pepsin, magnesium oxide (MgO) NPs, sodium alginate, gelatin, glycerol, sodium citrate, calcium chloride, sodium chloride, and sodium citrate dehydrate and all the other materials were purchased from Sigma Aldrich Corp. (St. Louis, MO, USA). The enzymes were stored at 4 °C and passed through a 0.45 μm filter prior to use.

2.2. Bacterial propagation and growth conditions

The stock solution of Li05 was maintained at −80 °C in MRS broth enriched with 50% glycerol. Li05 was propagated in 50 mL MRS broth at 37 °C for 24 h in a double chamber anaerobic hood with an airlock (88% N_2, 10% CO_2, and 2% H_2) purchased from Electrotek (West Yorkshire, UK). Cells were harvested by centrifugation (Eppendorf, Germany) at 4000 rpm/min for 10 min at 4 °C. The cell pellets were washed twice with buffer saline and re-suspended in 2 mL of buffer saline.

2.3. Preparation of microgels

Microgels were prepared according to the method we used previously with some modifications (Yao et al., 2017). Cells were proliferated in MRS broth in anaerobic chamber overnight and centrifuged to collect the bacteria. The cell pellet was suspended in 2 mL saline solution to reach a concentration > 10 $\log_{10}$ CFU/mL, which was then homogenized with 48 mL of biopolymer gelling solutions (mixture of 1% alginate (w/v) and 1% gelatin (w/v)) mixed with MgO NPs to form probiotic suspensions. The suspensions were then sprayed into 0.1 M calcium chloride solution using an electrostatic microencapsulation unit (NisoEngineering, Zurich, Switzerland) with a nozzle size of 300 μm. After hardening for 30 min, the microgels were collected using a sieve and rinsed three times with saline solution.

Samples of probiotic-loaded microgels were pre-frozen in a −80 °C freezer overnight and then lyophilized in a freeze dryer (Micromodulyo, Thermo Fisher, US).

2.4. Characterization of microgels

2.4.1. Optical and confocal microscopy

Wet microgels were observed by optical microscopy and images were recorded using a digital camera (C1 Digital Eclipse, Nikon, Tokyo, Japan). The location of bacteria in Li05-loaded microgels was determined using confocal fluorescence microscopy (LSM 710, Zeiss, Germany). After being labeled with fluorescence using a live/dead backlight bacteria viability kit (Thermo Fisher Scientific, US), the microgels were observed using an excitation wavelength of 485 nm and emission wavelengths of 530 nm (green) and 630 nm (red) separately. Flow cytometry (S3e™, Bio-rad, BD, US) was used to determine the ratio of live/dead Li05 cells in the microgels. The encapsulated cells were released by dissolving the beads in 10% sodium citrate. Then, the collected cells were stained using the live/dead backlight bacteria viability kit.

2.4.2. Scanning electronic microscopy (SEM)

Free Li05 were dehydrated using a critical point drying method as previously described (Yao et al., 2017). Freeze-dried microgels were then coated with a thin layer of gold by DC sputtering before being observed by an SEM (Hitachi SU8010, Tokyo, Japan).

2.4.3. Atomic force microscopy (AFM)

Microgel samples were applied to a mica sheet and the surface characteristics were then determined using an AFM instrument (Nanowizard, JPK, Germany) operated in the tapping mode. The root-mean-square (RMS) roughness and peak-to-valley roughness of the microgel surface was calculated as an average of four scan areas.

2.5. Viability of bacteria in harsh conditions

2.5.1. Long-term storage

Free cells and encapsulated bacterial-loaded microgels in physiological saline were stored in a plate at 4 °C for 4 weeks to mimic long-term storage of aqueous-based commercial products. The viability of the bacteria was detected each week using a plate count method: 1 g alginate beads was suspended in 9 mL of 10% sodium citrate dehydrate solution in order to dissolve the beads. The number of viable bacteria in the solution were determined by plate count on MRS agar though serial dilutions of the solution at 30 °C for 48 h.

2.5.2. Gastrointestinal conditions

Artificial simulated gastric fluid (SGF) and simulated intestinal fluid (SIF) were prepared according to previously described methods (Yao et al., 2017). The viability of the encapsulated bacteria was then determined using the following approach. A fixed volume (9 mL) of SGF or SIF was preheated in an incubator shaker (IKA KS4000i, Germany) operated at 37 °C and 100 rpm/min. Then, 1 mL of wet sample or 20 mg of freeze-dried sample was added to the fluids separately. The viability of the bacteria was monitored at different time points using the plate count method.

2.5.3. Heat treatment

The impact of encapsulation on the heat-resistance of the bacteria was determined by incubating the samples anaerobically at 63 °C. After heat treatment, the vials were cooled down in an ice bath immediately. Viable cell counts were enumerated using the same method as described above.

2.6. Statistic analysis

Experiments were repeated at least three times to determine the morphologies and surface properties of the microgels. The mean of 10 replicate drops was used to calculate cell viability. All values are expressed as the mean ± standard deviation (SD) unless stated