Development of a microencapsulation technique for probiotic bacteria *Lactobacillus casei* 431 using a protein-polysaccharide complex

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Abstract

According to the Food and Agriculture Organization (FAO) and the World Health Organization (WHO), probiotics are defined as ‘‘live microorganisms which when administered in adequate amounts confer a health benefit for the host’’ (FAO/WHO, 2001). *Lactobacilli* and *Bifidobacteria* are two major group of organisms considered to have probiotic properties. Probiotic bacteria are accepted universally for conferring beneficial effects to human gut health. However, the successful delivery of these bacteria to the human intestine via a proper food matrix is challenging because the stresses encountered by the probiotics during processing, storage and gastric transition cause major loss of viability. The primary objective of this study was to develop a novel protection system using a complexation product of dairy protein and a bacterial exo-polysaccharide which should be able to protect the probiotic bacteria during their gastric transit and also release them under suitable conditions in the intestine. *Lactobacillus casei* 431, a commercial strain from Chr Hansen, Denmark, was chosen as the experimental strain and the protein-polysaccharide complex was made up of sodium caseinate and gellan gum. Gelation of the sodium caseinate and gellan gum mixture was achieved by a gradual decrease of pH with slow hydrolysis of glucono-delta-lactone (GDL) and *Lactobacillus casei* 431 cells were successfully entrapped into this gel matrix. An intermediate step of forming a water-in-oil emulsion was involved in this process for producing micron level gel particles. The appropriate combination of ingredients, based on final elastic modulus to attain adequate gel strength, was finally decided as 10% (w/w) sodium caseinate, 0.25% (w/w) gellan gum and 2.5% (w/w) GDL. This combination resulted in a very fine and uniform capsule size distribution and up to 89% encapsulation efficiency was achieved.

The gelled microcapsules were freeze dried to obtain better shelf stability and easy handling properties. The particles obtained had diameters ranging from 40 to 1100 µm for wet and 46 to 631 µm for freeze dried microcapsules. The mean diameters (D_{32}) of wet and freeze dried microcapsules were found as 287 and 152 µm, respectively.

Scanning electron microscopic examination of the freeze dried particles showed irregular surfaces and the presence of numorous pores.

Tolerance of free and encapsulated bacterial cells in simulated gastric juice at pH 2.0 was tested in an *in vitro* model and the results showed better survivability of encapsulated cells in both wet and dry microcapsules as compared to the free cells. The log CFU reduction figures after a 2 hour incubation period, were 4.56 for free cells, 3.03 for cells inside wet capsules and 2.28 for cells protected inside freeze dried particles. Incubation of free and encapsulated cells in the presence of 1% (w/v) bile extract for 8 hours showed 2.51 log CFU/gm reductions for free cells with almost no detrimental effect on wet microencapsulated cells and 2.44 log CFU reductions for freeze dried cells.

Further research work was undertaken to improve the post freeze drying survivability of the *L. casei* 431 cells by including cryoprotective solutes in both the culture growth and the drying media. Trehalose and lactose were chosen as cryoprotecting agents. Compared to an average 1.70 log CFU reduction in case of control (no cryoprotectant) samples, trehalose and lactose containing samples both showed a much better survival rate; only 0.84 and 0.37 log CFU/gm reduction respectively, in cell population, were recorded. A membrane coating over the produced microcapsules was applied and the properties of such coated samples were checked separately. The coating process aided in the post drying survivability and only 0.53 and 0.13 log CFU/gm reductions were recorded for trehalose and lactose supplemented samples, respectively. The presence of cryoprotecting compounds proved to be beneficial against the simulated gastric environment and the membrane coating gave additional improvement in this regard. During the gastric fluid incubation tests, cryoprotected samples (freeze dried) containing trehalose and lactose shown a higher survival of 3.13 log CFU/gm and 2.04 log CFU/gm respectively, compared to cells in free form. Improvements offered by the membrane coating were recorded as an additional 0.23 log CFU/gm and 0.66 log CFU/gm higher survival for trehalose and lactose respectively. The same trend was observed for bile salt tolerance also. Cryoprotected samples (freeze dried) containing trehalose and lactose showed a higher survival of 0.41 log CFU/gm and 0.84 log CFU/gm respectively, compared to cells in free form. Additionally, the membrane coating process contributed

towards further improvement in viability of 0.25 log CFU/gm and 0.26 log CFU/gm for trehalose and lactose respectively. Overall, lactose has been found to be a marginally better protectant of cells than trehalose against freeze drying, acid and bile salt stresses.

The membrane coating process helped in forming a very smooth surface morphology devoid of any visible pores. Perhaps the presence of a membrane coating was responsible for this better protective nature of coated microcapsules. But as a drawback, this coating process resulted into higher particle mean diameters, both for wet and freeze dried beads.

Storage of freeze dried samples at 37°C proved to be more detrimental to the entrapped cells than at 4°C. But the results obtained were better compared to the situation where no protective compounds were used. It was found that lactose and trehalose helped in maintaining high levels of viable cell populations during the storage period but the cell degradation rate was positively correlated with the storage temperature.

Therefore, it can be concluded that a low pH sodium caseinate-gellan gum gel matrix can offer adaptation and protection to the probiotic cells before encountering a high acid stomach environment and therefore can be utilized as an effective microencapsulation technique. The survibility of the *L. casei* 431 cells could be further improved during freeze drying as well as gastrointestinal transit by incorporation of protectants, viz., lactose or trehalose and applying a membrane coating of gellan gum. High acid food preparations such as, yogurt and fruit juice could be the probable applications for the current findings.

To my wife Tuli, my son Antariksh and my parents

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