EFFECT OF ADDITION OF MALTODEXTRIN AND FRUCTOOLIGOSACCHARIDE IN LITCHI JUICE ON THE SURVIVAL OF MICROENCAPSULATED PROBIOTIC *L. PLANTARUM* MTCC2621 IN SIMULATED DIGESTIVE SYSTEM

6.1. Introduction

There is significant interest in the development of dietary supplements that benefit the composition and activity of health-promoting gut microflora. Probiotic survival during the processing and storage of functional food products is of concern for the development of products with a guaranteed content of bioactive cells [1]. Due to the fastidious nature of many probiotic bacteria, survival in sufficiently high numbers during passage through the human gastro-intestinal tract (GIT) remains a major challenge for effective delivery of these beneficial bacteria. Also, colonization of the intestine by an exogenous probiotic bacterium is influenced by many factors including the size of the inoculum, physiological state of the bacteria, buffering capacity of the delivery food and the capacity of the microorganisms to resist acid and bile encountered in the upper segments of the GIT [2]. The most common probiotic-containing foods are fermented dairy products that contain lactic acid bacteria (LAB). LAB, a group of commonly selected probiotics, are indigenous to the human intestine, where they preferentially colonise the colon [3]. The bacteria must therefore survive exposure to the acid in the human stomach and bile in the intestine in order to be effective [4].

Fruit and vegetable juices have been proposed as ideal delivery media for probiotic microorganisms [5] because they are rich in functional components, such as vitamins and antioxidants, and do not contain dairy allergens. Fruit drinks could serve as good probiotic carriers if precautions are taken in regards to their sensory characteristics [5] and pH. Probiotic bacteria lose viability during storage in many fermented milks having pH values between 4.0 and 5.0 [6]. Therefore, the even more acidic environment of some fruit juices, having pH values around 3.5, is of concern [7]. Although strain selection for addition to foods must be based on the sensory impact that the culture has on the final product [8], stability during storage and health benefits are of primary importance. It is desirable to have live cultures in the products [9], although there are instances where non-viable cells

have shown health benefits [10]. Probiotics should not only survive in the food product but also reach the small intestine alive.

Various solutions to this problem, such as durable strain selection in adverse environments [11] and addition of prebiotics [12], have been evaluated. Prebiotics are non-digestible dietary ingredients that benefit the host by selectively stimulating the growth and/or activity of beneficial bacteria in the colon [13], 1998). The most frequently studied examples are inulin-type fructans and fructooligosaccharides. Synbiotics, i.e., combination of probiotic and prebiotic agents improves the survival of bacteria in the upper gastrointestinal tract and enhance their effect in the large bowel [14,15,16]. Encapsulation has been investigated for protecting probiotics in food products and the gastrointestinal tract [17]; advantages include prevention of interfacial inactivation, stimulation of production and excretion of secondary metabolites, and continuous utilization. Additionally, encapsulation may enhance microbial survival and operating efficiency during fermentation [18] [19] and De Giulio et al. [20] found that encapsulation of LAB helped overcome inactivation during drying or exposure to artificial gastric conditions. Different biopolymers like maltodextrin, fructooligosaccharide (FOS), pectin, alginate, inulin, xanthan gum, pectin have been explored as encapsulation agent because these are non-toxic, biocompatible, and inexpensive [20].

Researchers have investigated the use of encapsulated LAB in dairy products [21,22,23,24] and used immobilized *L. acidophilus* to ferment banana puree and tomato juice and observed that the number of viable cells during fermentation had increased significantly relative to free cells. Incorporating prebiotics and bioploymers in coating materials may better protect probiotics in food systems and the gastrointestinal tract due to synbiosis [24, 25]

The goal of microencapsulation of probiotics is to protect microorganisms from adverse conditions, enabling the arrival in the intestine at the concentration required to exert its beneficial effect [26,27,28]. However, there is little information about using free and encapsulated microorganisms to ferment litchi juice and on their resistance to gastrointestinal stresses. The aim of this study was to evaluate whether microencapsulation of *L. plantarum* MTCC2621 with 10% (w/v) maltodextrin plus 5% (w/v)

fructooligosaccharide (FOS) (from results of Chapter V) affects the viability of probiotics when exposed to conditions simulating the passage through the gastrointestinal tract.

6.2. Materials and Methods

6.2.1. Probiotic strain and growth condition

Lyophilised *Lactobacillus* culture, *Lactobacillus plantarum* MTCC2621 was obtained from Microbial Type Culture Collection and Gene (MTCC) (Chandigarh, India). From this culture, stock solution was prepared by adding sterile glycerol (50% v/v). The glycerol stock culture was stored at frozen condition (-40 ^{o}C) in sterile screw cap tubes for future use. The probiotic organisms were grown individually by inoculating into 10 mL sterile de Man Rogosa and Sharp (MRS) broth [29] (Himedia Laboratories Pvt. Ltd, Mumbai, India) and incubated at 37 ^{o}C for 2 days under aerobic condition. The cells were harvested by centrifuging (Sigma, Germany) at 1500 x g for 15 min at 4^{o}C. Before inoculation into fruit juices, the harvested cells were washed twice with sterile saline water (0.85% w/v NaCl) to remove any residual MRS. The cell pellets were diluted to get a bacterial concentration of 10^{11} CFU/mL by saline water.

6.2.2. Preparation of fruit juice

Ripe and sweet variety of litchi (*Litchi chinensis* Sonn.) fruits that were purchased from the local fruit market, Tezpur, Assam during the season were peeled and pitted. The juice was extracted using a household juicer (Philips, Bangalore, India). The juice was strained through a muslin cloth and pasteurized at 90 ^{o}C for 1min with consistent stirring. Subsequently, the juice was cooled down to 25 ^{o}C. The pH and TSS (total soluble solids) of pasteurized juice was 3.65±0.26 of 14.6 ±0.3 ^{o}Brix, respectively. Litchi juice was selected for this study based on its suitability as probiotic carrier from studies reported in Chapter II.

6.2.3. Spray drying condition

Spray drying was performed using a spray drier (Lab Plant, UK) equipped with nozzle size of 0.1 mm. The drying condition of the experiment was maintained as per the optimized conditions mentioned in the Chapter IV. Pasteurised litchi juice was mixed with 10% (w/v) maltodextrin plus 5% (w/v) fructooligosaccharide (FOS) (coded as MF) which

had shown better results among all combinations in the previous chapter (Chapter V). The total soluble solids (TSS) of the litchi juice was adjusted to 11 °Brix (total solid concentration 0.1 g/L) by appropriate dilution with sterile distilled water because the pure extract was too viscous to be spray dried. Homogenization of this juice and coating material was done to obtain a bacterial concentration of 10^{11} CFU/mL using a magnetic stirrer (LaboTech) just before spray drying. The obtained powder was kept in an airtight container and stored at refrigerated condition (4±1°C) prior to simulation studies.

6.2.4. Simulated gastric juice (SGJ) and simulated intestinal juice (SIJ)

The simulated gastric and intestinal juices were prepared according to Mozzi et al. [30] and Picot and Lacroix [31], with modifications. The simulated gastric juice (SGJ) was prepared using potassium chloride (1.12 g/L), sodium chloride (2.0 g/L), calcium chloride (0.11 g/L) and potassium phosphate monobasic (0.4 g/L) followed by sterilization at 121 °C for 15 min. Mucin (3.5 g/L) and pepsin (0.26 g/L) were added to SGJ immediately before using and the pH was adjusted to 3.0 by adding 1N HCl. The simulated intestinal juice (SIJ) was prepared by adding pancreatin to the SGJ solution to obtain a final concentration of 1.95 g/L and the pH was then adjusted to 7.0 by adding 1N $NaHCO_3$. The viability of *L. plantarum* during simulated gastrointestinal conditions was measured using the methodology adapted from Krasaekoopt et al. [32] and Gebara et al. [33]. Freshly prepared spray dried powder (3 g) containing *L. plantarum* and MF was placed in a tube containing 30 mL of sterile simulated gastric juice (SGJ, pH 3.0) in 4 tubes and then incubated at 37 °C for 30, 60, 90 and 120 min in a shaking incubator (Sartorius, Germany) with a shaking speed of 150 RPM. After incubation, one tube is collected and encapsulates were removed by filtration and place in tube containing 9 mL of sterile simulated intestinal juice (SIJ, pH 7.0) containing pancreatin. The tubes were again incubated at 37 °C for 120 min and aliquots (1mL) were removed immediately after 30 min, 60 min and 120 min exposure, and assayed to evaluate the viability of *L. plantarum* in SIJ. Each treatment was performed in triplicate.

6.2.5. Bacterial enumeration

To determine the viable counts of *L. plantarum* after exposure to simulated gastric juice and simulated intestinal juice, the aliquots were re-suspended in 10 ml of phosphate buffer (0.1 M, pH 7.4), followed by gentle shaking for 30 min at room temperature.

Samples were taken at different time intervals to determine the complete release of encapsulated bacteria by plating on MRS agar. The colony forming units (CFU/ml) were determined by plating on MRS agar plates and incubation for 48 h at 37 ^{o}C. Free bacteria were enumerated on MRS agar. Peptone water was used to prepare the serial dilutions. The culture was plated using the pour plate technique and incubated at 37 ^{o}C for 48 h.

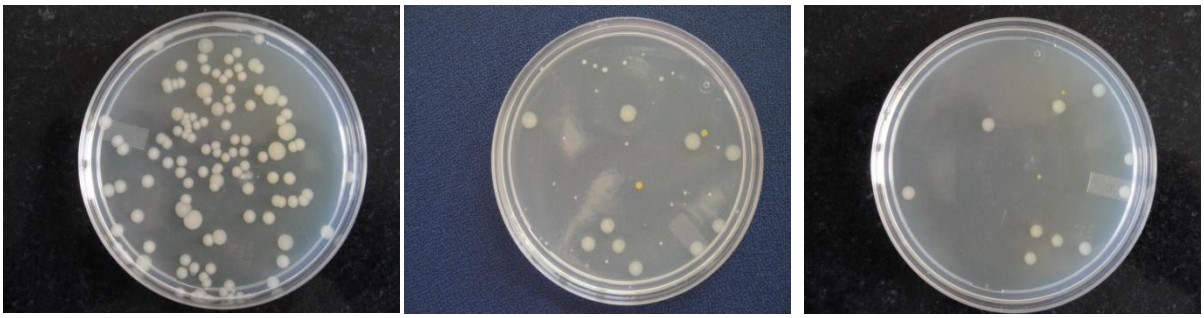
6.2.6. Statistical analysis

All experiments and analysis were done in triplicate. The results are expressed as mean ± standard deviation.

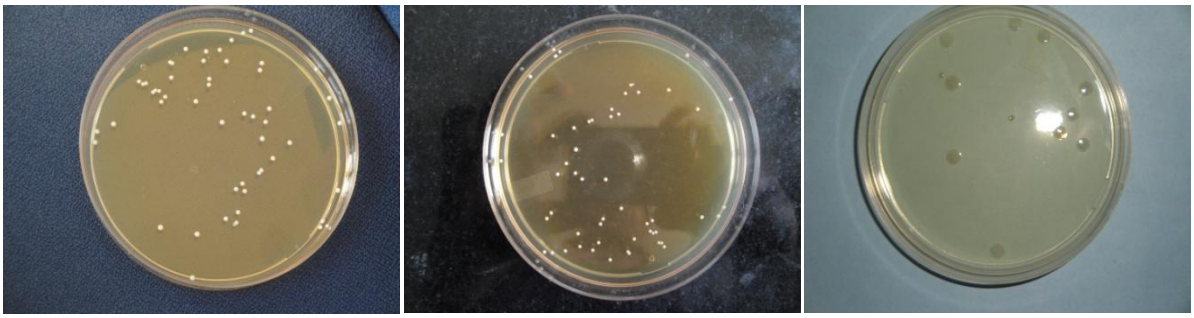
6.3. Results and Discussion

6.3.1. Survival of free and encapsulated *L. plantarum* in simulated gastric conditions

In order to exert positive health effects, probiotics should resist the stressful conditions of the stomach. Therefore, one main purpose of encapsulation is to improve the low pH tolerance of probiotics. The pH of gastric juices is about 1.5–3.0 [34].



SGJ-0 min SGJ-30min SGJ-120min



SIJ-0 min SIJ-30 min SIJ-120 min

Fig.6.1. Plates of encapsulated *Lactobacillus plantarum* MTCC2621 after exposure to simulated digestive system. SGJ: simulated gastric juice; SIJ: simulated intestinal juice

L. plantarum was found to be very sensitive to low pH. The viability of all free *L. plantarum* was lost dramatically when it was exposed to gastric conditions [35]. As many investigations have reported, most of free probiotics are easy to be damaged by stomach acid. Sohail et al. [36] reported more than 6 log CFU/mL of *L. plantarum* was lost in pH 3.0 of SGJ after exposure for 20 min. Encapsulation of *L. plantarum* by spray drying with maltodextrin and fructooligosaccharide (FOS) had positive effect on the survival of *L. plantarum* in SGJ as shown in **Table 6.1**. At pH 3.0 of SGJ, viability of *L. plantarum* encapsulated with maltodextrin plus fructooligosaccharide (FOS) was fully maintained even after 120 min incubation (**Fig 6.1**).

Table 6.1. Viability of *Lactobacillus plantarum* MTCC2621 (log10 CFU/g) in litchi juice with or without encapsulation in maltodextrin plus fructooligosaccharide during exposure to simulated gastric juice (SGJ, pH 3.0) and simulated intestinal juice (SIJ, pH 7.0) for 120 min (n=3)

Time	Free (log_{10}cfu/g)			Encapsulated (log_{10}cfu/g)		
(min)	Control	Post-SGJ	Post-SIJ	Control	Post-SGJ	Post-SIJ
0	9.34±0.27	6.60±0.21	2.74±0.20	6.45±0.12	6.11±.028	5.36±0.19
30	8.80±0.12	4.22±0.17	3.30±0.44	6.06±0.17	5.90±0.04	5.60±0.10
60	8.33±0.21	3.45±0.30	3.72±0.19	5.88±0.53	5.74±0.25	5.65±0.14
90	8.04±0.11	3.11±0.34	4.11±0.08	5.80±0.29	5.22±0.17	5.71±0.08
120	7.75±0.30	2.91±0.18	4.15±0.04	5.55±0.34	5.15±0.09	5.80±0.20

Thus, microencapsulation conferred protection to *L. plantarum* during exposure to simulated gastric juice at pH 3.0. Moreover, coating of *L. plantarum* with maltodextrin plus fructooligosaccharide conferred additional protection to *L. plantarum* by preventing diffusion of acidic groups and enzymes into the particles [37], affecting the viability of probiotics before and after coating in a similar way. After 120 min exposure to simulated gastric juice at pH 3.0 the viability of free cells as well as encapsulated (*L. plantarum* + MF) was 2.91 and 5.15 (log10 CFU/mL), respectively. As described by Krasaekoopt et al. [32], coating provided better protection to *L. acidophilus* 547 and *L. casei* as compared to uncoated particles. Coating with maltodextrin and fructooligosaccharide conferred greater protection to *L. plantarum* when exposed both to simulated gastric juice (pH 1.8 in the presence of pepsin) and simulated intestinal juice (pH 6.5, in the presence of pancreatin,

trypsin and bile salts) when compared to the particles without coating [38]. Coating maltodextrin with fructooligosaccharide also conferred greater protection to *L. rhamnosus* CRL 1505 when exposed to acidic condition (pH 2.0 in the presence of mucin and pepsin) as compared to free microorganisms [33], but the survival of the microorganisms in the uncoated particles was not evaluated. The results presented in this work corroborate other studies in literature that demonstrate the efficiency of microencapsulation in protecting probiotic microorganisms during exposure to gastrointestinal conditions [32,33,38].

Fructooligosaccharide is approved for use in the food industry and it has a positive effect on human health [39]. Researchers have reported the positive effects of fructooligosaccharide on cell survival in simulated gastrointestinal system. Sultana et al. [22] showed that the addition of starch to an alginate mixture increased recovery of encapsulated cells; however, they remained sensitive to acid. Chen et al. [16] used fructooligosaccharide and sodium alginate to microencapsulate different probiotics and obtained high survival. Chan and Zhang [40] tested the ability of a similar matrix to protect probiotic cells in the human digestive tract, achieving a remarkable increase in survival under acidic conditions relative to free cells.

6.4. Conclusion

The number of free cells dramatically dropped in the first 30 min due to the acidic effect of gastric juice; and then constantly decreased until the end of the study period (120 min). After 120 min, when the same cells were exposed to simulated intestinal environment the number of cells increased due to better environment and high pH of intestinal fluid. Microencapsulation of probiotics with maltodextrin and fructooligosaccharide in litchi juice not only provided better protection to probiotic bacterial cells from the harsh conditions of gastric environment, but also enhanced the growth of these microorganisms in simulated digestive system. The synbiotic effect of coating material, probiotics and the delivery medium, therefore, enhances the survivability of probiotics in gut environment.

6.3. Bibliography

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