

Effect of added prebiotic (Isomalto-oligosaccharide) and Coating of Beads on the Survival of Microencapsulated *Lactobacillus rhamnosus* GG

Sing Chee SIANG¹, Lai Ka WAI¹ , Nyam Kar LIN¹, Pui Liew PHING^{1*}

Abstract

The study aimed to encapsulate *Lactobacillus rhamnosus* GG (LGG) with the selected prebiotic, using co-extrusion technology with a poly-L-lysine (PLL) coating and evaluate probiotic survival in simulated gastrointestinal conditions. Selection of ideal prebiotic was conducted using inulin, fructo-oligosaccharide (FOS) and isomalto-oligosaccharide (IMO) and its optimal concentration to be incorporated in microencapsulation was determined. Microcapsules without coating (S₁: no prebiotic and S₃: with prebiotic) and with coating (S₂: no prebiotic and S₄: with prebiotic) produced were evaluated based on its physical properties and survival in simulated gastrointestinal environment. The IMO with a concentration of 3.0% (w/v) was selected due to its best effect in promoting growth of LGG after 24 h (8.63±0.07 log CFU/mL). The morphology analysis revealed that all microcapsules produced were spherical with a diameter ranging from 491.3 to 541.7 µm and microencapsulation efficiency ranged from 84.16 ±5.30% to 90.56±3.33%. The incorporation of IMO and coating with PLL improved the survival of LGG by 3% up to 52% after 2 h of incubation in simulated gastric digestion. Among all formulations, PLL coated microcapsules added with IMO was the most effective in protecting LGG during the first hour of simulated gastric digestion (6.52 log CFU/mL) with cell viability greater than the minimum recommended level of 10⁶ CFU/mL.

Keywords: microencapsulation; probiotic; co-extrusion; isomalto-oligosaccharide; poly-L-lysine; *Lactobacillus*.

Potential Applications: The use potential coating materials and prebiotic in probiotic microencapsulation that can retain high survivability in simulated gastrointestinal conditions and can be further incorporate in food products.

1 Introduction

Probiotics are defined as living microorganisms that, when administered in adequate amount, confer a health benefit on the host (Hill et al., 2014). Lactic acid bacteria (LAB), mainly from the genera of *Lactobacillus* and *Bifidobacterium*, possess probiotic characteristics, such as acid and bile tolerance, antimicrobial effect against pathogenic bacteria, and ability to adhere onto mucosal and epithelial surfaces (Kechagia et al., 2013; Ranadheera et al., 2018). Among probiotics, *Lactobacillus rhamnosus* GG (LGG) is one of the well-studied LAB that was first discovered by Sherwood Gorbach and Barry Goldwin from healthy adults' fecal samples (Gorbach et al., 2017). LGG is a rod shaped, non-spore forming and facultative gram positive bacteria which is categorized as generally recognized as safe (GRAS) (Valík et al., 2008; Kailasapathy, 2014). It has been extensively studied and applied in dairy products such as cheese, milk and yogurt, with study on increasing the buttery flavoring compounds following different sugar substrate incorporation (Assaf et al., 2019; Bang et al., 2014; Jyoti et al., 2003). The health benefits of LGG includes the prevention or treatment of gastrointestinal infections and diarrhea, stimulating the immune responses and preventing certain allergic symptoms (Berni Canani et al., 2012; Fong et al., 2015; Segers & Lebeer 2014).

According to Gibson et al. (2017), prebiotic is a substrate that is selectively utilized by host microorganisms conferring a health benefits. Most of the previously studied prebiotics were

shown to stimulate the growth and activity of colon microflora (Patel & Goyal, 2012; Vrese & Schrezenmeir, 2008; Khosravi Zanjani et al., 2014). Incorporation of prebiotics with probiotics produces a synergistic health beneficial effect to human, termed as "synbiotics" (Sarao & Arora, 2015). Several studies had revealed that microencapsulation of probiotics with prebiotics resulted in protection on viability of probiotics during gastrointestinal transit (Crittenden et al., 2006; Khosravi Zanjani et al., 2014). IMO are glucose oligomers that can be found naturally in many fermented foods such as miso and sake. Meanwhile, commercial IMO is produced enzymatically through transglycosylation using starch (Niu et al., 2016; Sawangwan & Saman 2016). IMO is only partially digested by human and the undigested portion will be fermented by *bifidobacteria* in the colon which improve the intestinal flora (Mao et al., 2015; Wang et al., 2015). Recent studies also demonstrated that the incorporation of IMO into cheese and fermented beverage enhance their functional properties (Liu et al., 2015; Mei et al., 2017).

The minimum amount of probiotics required to exert health benefits to hosts is in the range 10⁶ – 10⁷ CFU/mL (Food and Agriculture Organization & World Health Organization, 2002; Kechagia et al., 2013). In order for probiotic to survive harsh conditions such as low pH and high bile concentration during digestion, microencapsulation is one of the common techniques that can be applied to protect the probiotics against these adverse

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¹Department of Food Science with Nutrition, Faculty of Applied Sciences, UCSI University, Kuala Lumpur, Malaysia

*Corresponding author: puihp@ucsiuniversity.edu.my

environments and controls the release of probiotic in human using certain encapsulation materials (Ozyurt & Ötles, 2014; Shori, 2017; Nogueira et al., 2017). Microencapsulation produces tiny particles of liquid or solid materials surrounded or coated with a continuous film of inert polymeric material (Etchepare et al., 2015). The materials encapsulated in the internal of capsule is known as core, while the wall is known as shell or coating (Suganya & Anuradha, 2017). This technique has been successfully protect the active ingredient such as red beet extract, grape seed oil, and linalool under unfavourable conditions (Antigo et al., 2018; Böger et al., 2018; Xiao et al. 2017).

Alginate is among the commonly used wall materials for microencapsulation (Colom et al., 2017). D-mannuronic acid and L-glucuronic acid present in alginate has the ability to form a stable gel (Etchepare et al., 2015). The structure of capsules is formed through cross linking in the presence of calcium ions from calcium chloride (Martin et al., 2014). However, this network can be destabilized by chelating agents such as lactates, citrates and phosphates (Leirvag, 2017; Chew et al., 2019). Therefore, it has been suggested that alginate beads should be coated with other wall material to reduce the porosity of alginate matrix to improve the physical integrity to prevent leakage of probiotics (Sarao & Arora, 2015). Poly-L-lysine (PLL) forms a strong network between the free amine groups of PLL and uronic acid of alginate that could increase the resistance of capsules toward chelating agents. This enhances the mechanical strength of capsules (King et al., 2003; Solanki et al., 2013; Zanjani et al., 2017).

Several researches have been conducted on encapsulation of probiotics using emulsion, spray drying and extrusion methods (Anekella & Orsat, 2013; Krasaekoopt & Watcharapoka, 2014; Mandal & Hati, 2017). Microencapsulation by vibrating technology or commonly known as co-extrusion is a promising encapsulation technique as it can produce uniform and smaller size capsules compared to extrusion technique (Krasaekoopt et al., 2003; Nemethova et al., 2014; Sri et al., 2012). Yet, few studies have applied co-extrusion technique to encapsulate probiotic, in comparison with extrusion technique that are commonly used (Olivares et al., 2017; Silva et al., 2016).

Although LGG is associated with many beneficial effects on health and prevention of disease in human. However, there are limited studies focusing on the incorporation of IMO as part of the core in probiotic microencapsulation and the survivability of these microcapsules in harsh conditions along the gastrointestinal tract (Cook et al. 2012). Therefore, the aim for this study is to investigate the effectiveness of microencapsulation on the survivability of probiotic during gastrointestinal transit by the addition of prebiotic and coating with PLL.

2 Materials and methods

2.1 Preparation of culture

Preparation of LGG culture was adapted from Cheng (2015) with modification. A sachet (2g) of LGG (LactoGG, USA) was added to 100 mL MRS broth (Chemsoln, India) and incubated for at 37 °C for 24 h. After sub-cultured twice, LGG was cultivated in 100 mL of MRS broth and incubated at 37 °C for 24 h. The cells were then harvested by centrifugation (5840 R, Eppendorf) at 6000x g, 4 °C for 10 mins, followed by resuspension of cell in 25 mL of Phosphate Buffer Saline (PBS).

2.2 Selection of prebiotics to microencapsulate LGG

Inulin (Sensu, Netherlands), Fructo-oligosaccharide, FOS (Sensus, Netherlands), or Isomalto-oligosaccharide, IMO (CK Chemicals, Malaysia) was added to 100 mL of MRS broth at 3.0% (w/v) and autoclaved. Sterile MRS broth was used as control. Active culture of 1.0% (v/v) was inoculated into the media supplemented with the tested prebiotic and incubated at 37 °C for 2 h. Enumeration of LGG were carried out by pour plate method (incubate at 37 °C for 48 h) using sterile MRS agar (Chemsoln, India). Aliquots (1 mL) was withdrawn from sample, serially diluted and pipetted onto the Petri dish. The viable cell counts for microcapsules and free cells were calculated using Equation 1 and expressed as logarithm colony forming unit per milliliter (log CFU/mL):

$$\text{Viable cell count (CFU/mL)} = \frac{\text{mean number of colonies}}{(\text{dilution factor} \times \text{volume plated})} \quad (1)$$

2.3 Optimizing concentration of selected prebiotic prior to microencapsulation

Selected prebiotic was incorporated into 50 mL MRS broth at different concentrations (0 – 4.0% with interval of 0.5% (w/v)). The samples were sterilized and inoculated with 1.0% (v/v) of active LGG culture. The samples were incubated at 37°C and enumeration of viable cell count was carried out after 24 h.

2.4 Microencapsulation of LGG using co-extrusion technique

Microencapsulation of LGG was carried out by co-extrusion using Büchi encapsulator B-390 (shown in Figure 1) as described by Chew et al. (2015) with modifications. The core fluid (LGG with or without IMO) and shell fluid (1.5% (w/v) sodium alginate (R&M Chemicals, UK) were pumped simultaneously through the concentric nozzles with diameter of 200 µm (inner nozzle) and 300 µm (shell nozzle), respectively, to give a core shell fluid stream. Dispersed droplets were hardened in 3% (w/v) calcium chloride (R&M Chemicals, UK) solution for 30 mins. The air pressure (600 mbar), vibration frequency (300 Hz), voltage (1.5kV) and amplitude of 3 was fixed for every encapsulation.



Figure 1. Büchi encapsulator B-390.

2.5 Coating with Poly-L-lysine

Alginate microcapsules (15 g) produced was coated with 0.05% (w/v) PLL (VIS Foodtech, Malaysia) solution by agitation at 100 rpm for 60 mins as described by Zanjani et al. (2017). Four formulations of LGG beads were produced without PLL coating (S_1 : no prebiotic and S_3 : with prebiotic) and with PLL coating (S_2 : without prebiotic and S_4 : with prebiotic).

2.6 Morphology and size of bead and Microencapsulation Efficiency (MEE)

The morphology and mean diameter of 10 randomly selected capsules were determined and measured using an optical microscope (CX31, Olympus, Japan) at 10x magnification and a stage micrometer (Chia et al., 2015). The solubilisation of co-encapsulated LGG cells was adapted from Yeung et al. (2016) with modifications. For decomposition of the capsules, 1 g capsules were transferred to 9 mL of 10.0% (w/v) sterile trisodium citrate (Merk, KGaA, Germany) to dissolve. Aliquots (1 mL) were withdrawn, serially diluted and enumeration of the beads were evaluated by pour plate method incubation at 37 °C for 48 h. Microencapsulation efficiency (MEE) was then calculated using Equation 2 (Lotfipour et al., 2012):

$$\text{MEE (\%)} = \frac{\text{Log}_{10}(\text{number of viable entrapped cells released from capsules})}{\text{Log}_{10}(\text{number of free viable cells in culture})} \times 100 \quad (2)$$

2.7 Sequential digestion

Sequential digestion was adapted from Ramos et al. (2016) with slight modifications. To evaluate the survivability of LGG under simulated gastrointestinal condition, 1 g of capsules or 1 mL of free cells were added to 9 mL of sterile simulated gastric juice (SGJ: 7 mL/L 0.1 M HCl, 2 g/L NaCl, 3.2 g/L pepsin) at pH 2.0 and incubated at 37 °C for 0 – 2 h (with interval of 1 h) with constant agitation at 100 rpm. After gastric digestion, the capsules or free cells were immediately adjusted to pH 6.8 with 1.0 M NaOH to inactivate pepsin. SGJ was removed by centrifugation at 6000x g, 4 °C for 10 minutes. After incubation in SGJ, 9 mL of simulated intestinal juice (SIJ: 6.8 g/L KH_2PO_4 , 190 mL/L 0.1M NaOH, 6 g/L bile salt) at pH 7.5 was added into capsules or free cells and incubated at 37 °C for 1 h and 2 h with constant agitation at 100 rpm in water bath. For retrieval of capsules or free cells after incubation, the mixture was centrifuged at 6000x g, 4 °C for 10 minutes. The capsules were released by adding 9 mL of 10.0% (w/v) trisodium citrate. For free cells, the cell pellet obtained from centrifugation was resuspended in PBS and the enumeration were determined by pour plate method.

2.8 Statistical analysis

All analyses were conducted in triplicate and the results were expressed as mean \pm standard deviation. One way analysis of variance (ANOVA) was carried out and Tukey's HSD test was used to determine the significant difference with $p \leq 0.05$ using MINITAB 19 (Minitab LLC, USA).

3 Results and discussion

3.1 Selection of prebiotic to microencapsulate with LGG

Preliminary screening of prebiotics were carried out before microencapsulation to select the optimum prebiotic for production of synbiotic capsules with LGG. The effects of inulin, FOS and IMO on growth of LGG are shown in Table 1 and it shows that all three prebiotics (inulin, FOS and IMO) had higher viable cell count of LGG compared to control (MRS broth without prebiotic). These results indicated that all prebiotics improved the growth of LGG similarly. Inulin and IMO could promote the growth of LGG (Chen et al., 2011; Succi et al., 2017). On the other hand, results shown in Table 1 indicated that FOS could promote growth of LGG. The above findings contradict the study conducted by Kaplan & Hutkins (2000). Their study demonstrated that LGG is unable to utilize FOS as energy source. In addition, Watson et al. (2013) also reported that FOS is more effective in promoting growth of *bifidobacteria* sp. instead of *lactobacillus* sp.

Since all prebiotic has the same effectiveness in promoting growth of LGG, the prebiotic is selected based on the least studied prebiotic. Inulin is a well-studied prebiotic which has been reported to enhance survivability of LGG during storage and gastrointestinal transit (Soukoulis et al., 2014; Atia et al., 2016; Gandomi et al., 2016; Seyedain-Ardabili et al., 2016). However, FOS, it was reported to be more bifidogenic and has little effect on *Lactobacillus* species. Therefore, IMO is the prebiotic selected for further analysis as it is known as emerging prebiotic and was less studied compared to inulin (Stowell, 2007).

3.2 Optimizing the concentration of IMO Prior to Microencapsulation

The optimization on the concentrations of IMO was carried out before co-encapsulated with LGG as the core material. Table 2 shows the effect of concentration of IMO

Table 1. Effect of different prebiotics on growth of LGG.

Prebiotics	Viable cell count (log CFU/mL)
Control (without prebiotic)	8.52 \pm 0.04 ^a
Inulin	8.83 \pm 0.03 ^b
Fructo-oligosaccharide	8.77 \pm 0.06 ^b
Isomalto-oligosaccharide	8.77 \pm 0.04 ^b

^{a-b}Means \pm standard deviations followed by different superscript letters within the same column are significantly different at $p \leq 0.05$ according to Tukey's test.

Table 2. Effect of IMO concentrations on growth of LGG.

Concentration of Isomalto-oligosaccharide (% (w/v))	Viable cell count (log CFU/mL)
0	8.40 \pm 0.04 ^a
0.5	8.34 \pm 0.04 ^a
1.0	8.37 \pm 0.06 ^a
1.5	8.44 \pm 0.05 ^{ab}
2.0	8.50 \pm 0.06 ^{ab}
2.5	8.55 \pm 0.05 ^{ab}
3.0	8.63 \pm 0.07 ^b
3.5	8.56 \pm 0.02 ^b
4.0	8.58 \pm 0.10 ^b

^{a-b}Means \pm standard deviations followed by different superscript letters within the same column are significantly different at $p \leq 0.05$ according to Tukey's test.

on the growth of LGG. From Table 2, it was observed that increasing concentration of IMO from 1.0 to 3.0% (w/v) increased the growth of LGG by 3%. LGG is able to hydrolyse IMO into D-glucose by the oligo 1-6 glucosidase enzymes. Therefore, at higher prebiotic concentration, higher carbon source is available for utilization by LGG (Goderska et al., 2008; Soto, 2013). Corcoran et al. (2005) reported that presence of metabolizable sugar can enhance the survivability of LGG in acidic environment, in which IMO can be metabolized by LGG (Soto, 2013). However, further increase in IMO concentration (from 3 to 4% (w/v)) did not improve the growth of LGG. Since no study has examined the effect of IMO concentration on specific strain of LGG, therefore, 3% (w/v) IMO was incorporated into the microencapsulation of LGG.

These results were in agreement with Chen et al. (2011), which reported that no significant difference ($p > 0.05$) in the growth of LGG was observed when IMO concentration ranging from 0.1% to 1.0% (w/v) was used, while contradicts with our findings that 2.0% (w/v) IMO would inhibit the growth of *L. rhamnosus*. In addition, the highest concentrations of IMO

used in their study were 2.0% (w/v). The possible reason could be due to the different *Lactobacillus rhamnosus* strain used which is not mentioned in the study. Similarly, Liu et al. (2015) reported that 1.0% (w/v) IMO promoted viability of *Lactobacillus rhamnosus* 6134.

3.3 Morphology, size and microencapsulation efficiency of bead

Figure 2 shows the microscopic evaluation on shape and size of four different capsules (S_1 , S_2 , S_3 , and S_4) whereas the average size of capsules and microencapsulation efficiency (MEE) were presented in Table 3. All capsules (S_1 , S_2 , S_3 , S_4) produced were white in colour. Under microscopic evaluation, Figure 2b and d show that the capsules coated with PLL has a very thin layer of membrane surrounding them, which is consistent with the findings of Zanjani et al. (2017). This coating can reduce the destabilizing effect of chelating agents on the structure of capsules (Krasaekoopt et al., 2004). In addition, PLL coating on alginate capsules also reduces the clumping between capsules (Cook et al., 2012). The microscopic evaluation shows that all capsules produced are spherical, uniform in shape and have

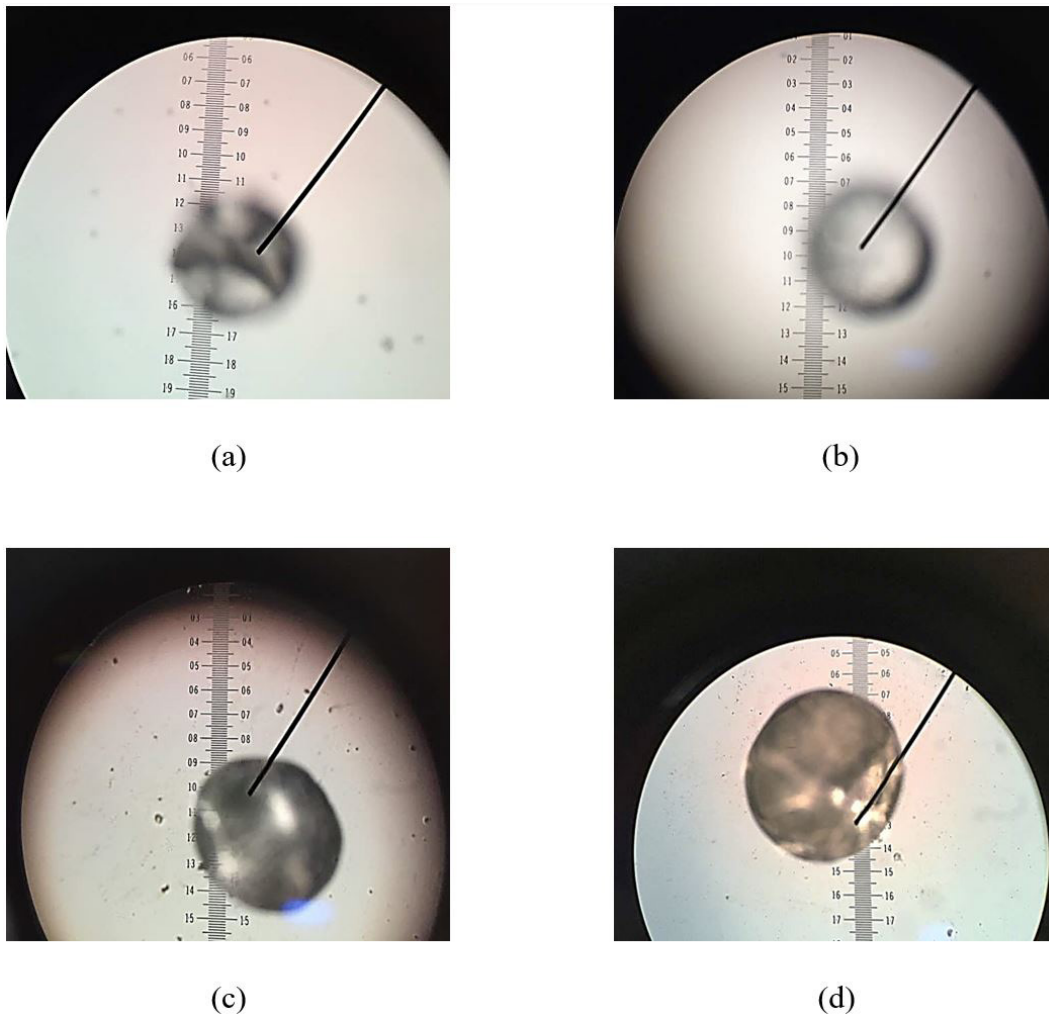


Figure 2. Shape and size of capsules measured with a scale micrometer. (a) S_1 (without IMO without PLL), (b) S_2 (without IMO with PLL) (c) S_3 (with IMO without PLL) (d) S_4 (with IMO with PLL).

Table 3. Average capsules size in diameter and MEE (%) of LGG.

Samples	Prebiotic	Wall material	Diameter (μm)	MEE (%)
S ₁	-	Calcium alginate	491.33 \pm 14.47 ^a	90.56 \pm 3.33 ^a
S ₂	-	Calcium alginate - PLL	498.33 \pm 27.50 ^a	84.16 \pm 5.30 ^a
S ₃	IMO	Calcium alginate	526.33 \pm 5.51 ^{ab}	87.39 \pm 5.25 ^a
S ₄	IMO	Calcium alginate - PLL	541.67 \pm 7.02 ^b	90.38 \pm 1.59 ^a

^{a-b}Means \pm standard deviations followed by different superscript letters within the same column are significantly different at $p < 0.05$ according to Tukey's test.

smooth surface. These results revealed the ability of co-extrusion technique to produce uniform capsules (Homar et al., 2007).

Table 3 shows that the size of the capsules produced were in the range of 491.33 – 541.67 μm . In comparison between S₁ and S₃, there was no significant difference ($p > 0.05$) in the mean diameter, whereas there was significant difference ($p \leq 0.05$) in mean diameter in comparison between S₂ and S₄. This result demonstrated that the size of the capsules was not dependent when IMO is added in the capsules and this was in agreement with the results from Zanjani et al. (2017). In comparison between S₁ and S₂, there was no significant difference ($p > 0.05$) in capsules size when the beads are coated with PLL. Similar findings are also observed in comparison between S₃ and S₄. These results were in contrast with Krasaekoopt et al. (2004) and Zanjani et al. (2017), which both reported coating of capsules with PLL increased the size of capsules significantly ($p \leq 0.05$). However, S₄ produced largest capsules compared to all formulations due to the synergistic effect of the addition of IMO together with coating using PLL.

However, there was no significant difference ($p > 0.05$) observed between the MEE for the four formulations. This showed that the addition of IMO and coating of PLL did not affect the MEE of LGG. The present finding also supported the study conducted by Zanjani et al. (2017). The MEE obtained ranged 84.16% to 90.56%, the high MEE values could be due to the gentle method that was used for microencapsulation (Krasaekoopt & Watcharapoka, 2014). Co-extrusion technique consumes less time as compared to manual encapsulation and with higher encapsulation efficiency during scale up production, in terms of speed and uniformity of the beads size. Besides that, this process could be carried out under non-toxic and sterile conditions with high production rate of uniformly microcapsules (Chew et al., 2015). These characteristics are vital for mass production. The probiotic microcapsule incorporated with IMO also exhibited a high MEE which is suitable for further application in food industry such as yogurt, cheese, fruit juice, herbal tea and cereal (Champagne et al., 2018; Heydari et al., 2018; Murtaza et al., 2017; Ng et al., 2019; Wong et al., 2019).

3.4 Sequential digestion for free and encapsulated LGG with different formulation

Figure 3 shows the viability of free cells and microencapsulated LGG under sequential digestion. It was observed that the viability of free cells and all encapsulated LGG (S₁, S₂, S₃, and S₄) decreased throughout the gastrointestinal digestion with the lowest viabilities presented by free cells. Various enzymes, acidic pH and presence of bile will threaten the viability of probiotic

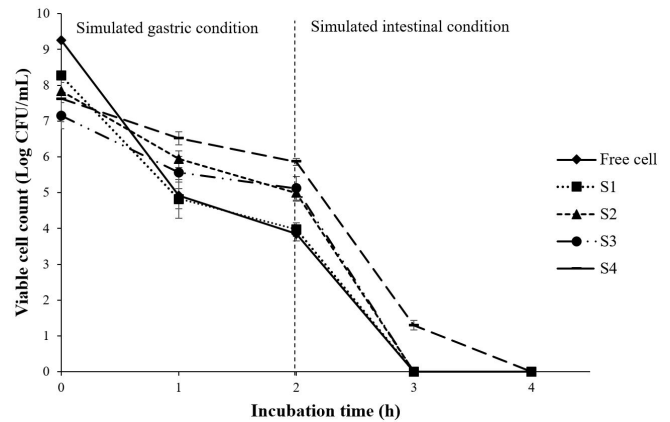


Figure 3. Average log CFU/mL of free cell, encapsulated LGG under sequential digestion. (S₁: without IMO without PLL; S₂: without IMO with PLL; S₃: with IMO without PLL; S₄: with IMO with PLL).

(Cook et al., 2012). When free LGG cells were sequentially incubated in SIJ for one hour, the survivability of LGG reduced drastically ($p < 0.05$) to 0% and this demonstrated that free cells had high sensitivity of LGG towards simulated gastrointestinal condition (Burgain et al., 2013; Li et al., 2016). In contrast, all encapsulated LGG had higher survival rate than free LGG cells during the first and second hour of SGJ incubation, which indicated that microencapsulation improved the survivability of LGG in gastric condition. Among the four different capsules produced, S₁ (uncoated bead without prebiotic) is the least effective in improving the acid tolerant of LGG due to the porous surface of alginate capsules which allowed the SGJ to enter into the capsules (Mortazavian et al., 2008; Shori, 2017).

From Figure 2, higher survivability rate of LGG was observed in S₂ compared to S₁ by 17.44% for the first hour and 15.61% for the second hour. This indicated that the presence of PLL coating on LGG beads could preserve viability of bacterial cells in SGJ. Formation of complex between the free amines in PLL and uronic acid in alginate which reduce the porosity of alginate capsules this increase the physical integrity of the encapsulating matrix (Lee & Mooney, 2012; Ding & Shah, 2008). Similar results were also presented by S₃ such that the LGG survivability was retained by at least 70% for both first and second hour as compared to S₁. These results suggested that IMO could protect LGG in SGJ incubation as it can serve as the carbon source for LGG and this was supported by findings from Corcoran et al. (2005) and Soto (2013).

Furthermore, when comparing S_3 with S_2 , the results showed that S_3 had lower mortality rate of LGG at second hour incubation in SGJ, this indicated that IMO had better effect in preserving viability of LGG compared to PLL. In contrast, S_4 achieved the highest survivability with more than 30% for the simulated gastric digestion as compared to other formulations. The highest acid tolerance of LGG in S_4 could be due to the synergistic effects between reduction of porosity in alginate capsules by coating material and protective effect of prebiotic on LGG simultaneously (Krasaekoopt & Watcharapoka, 2014; Zanjani et al., 2017). The survivability of free LGG cells and in all types of capsules decreased drastically to 0 log CFU/mL after the first hour in SIJ except for S_4 with at least cell viability of 1 log CFU/mL. However, no viable cell was observed at the second hour in of SIJ digestion. These findings suggested that the alginate beads coated with PLL and the addition of prebiotic can preserve the viability of LGG in SGJ digestion but only for the first hour of incubation in SIJ.

The weakness of PLL in protecting LGG in SIJ can be due to swelling of capsules in SIJ as shown in Figure 3 and subsequently reduced in structural integrity (Islam et al., 2010). The weak in structure may be due to forming of alginate/PLL complex with high guluronic content alginate that has resulted in random coil conformation, which is weaker than the helical formation between PLL with high mannuronic content alginate (Constantinidis et al., 2007). This study has shown that IMO and PLL can enhance survivability of LGG in acidic condition, but is not effective in preserving viability of LGG in intestinal condition.

4 Conclusion

This study demonstrated that IMO is potential prebiotic source for *Lactobacillus rhamnosus* GG as it was able to promote its growth similarly with other commercial prebiotics. The morphologies and the encapsulation efficiency of the microcapsules were not affected by the addition of IMO or the coating of PLL alone. However, the LGG microcapsules incorporated with IMO and coating of alginate beads with PLL increased the beads sizes with high microencapsulation efficiency. The alginate microcapsules coated with PLL and the addition of IMO could preserve the survivability of LGG in simulated gastric digestion but insufficient in simulated intestinal conditions. Hence, further studies such as using different combinations of wall materials or coating material are suggested to improve the porosity of the alginate microcapsules so that the probiotic cells can be protected from the adverse environment in intestinal conditions.

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