The aim of the study was to evaluate the adherence potential of indigenous probiotic bacteria and to improve the gastrointestinal survival of these cultures by adopting the double microencapsulation technique. The mean with standard deviation of triplicate experiments for the cell surface hydrophobicity, aggregation, and cell adhesion evaluation of indigenous probiotics revealed that there was no significant difference in the hydrophobicity of both solvents (n-hexadecane and Xylene). A mixed trend was observed in the estimation for hydrophobicity; the indigenous Lactobacillus acidophilus was found with highest cell surface hydrophobicity (56.3%) and the lowest was found in Lactobacillus reuteri (28.1%). The Ca-alginate and prebiotics amalgum was used in double treatment and compared with control (free) and single encapsulated (Ca-alginate) cells in the stimulated gastric juice (SGJ) and stimulated intestinal juice (SIJ). The one-way analysis of variance (ANOVA) results show that the double microencapsulation technique has significant effects (P< 0.05) on the survival of bacterial cells during 6 weeks storage. A negligible reduction was found on day 42 in case of double microencapsulated cells as compared to significant adverse effects on the free cell. The loss was higher in single microencapsulated Lactobacillus plantarum and Lactobacillus paracasei and zero loss for Lactobacillus delbrueckii subsp. Bulgaricus. While a slight revival was observed in the free and single encapsulated bacteria in SIJ. Thus, combination of Ca-alginate and prebiotics significantly improves the viability and stress response of probiotics in the harsh GI conditions.

Key words: Surface-hydrophobicity, aggregation, adhesion, double- microencapsulation, stimulated gastric Juice, stimulated intestinal juice, prebiotics, probiotics.

INTRODUCTION

Foods are no longer considered by consumers only in terms of taste and immediate nutritional needs, but also in terms of their ability to provide specific health benefits beyond their basic nutritional value. Currently, the largest segment of the functional food market is provided by the foods targeted towards improving the balance and activity of the intestinal microflora (Saarela et al., 2002). The nutritional effects due to the presence of probiotics in the products led Ilya Metchnikoff towards Nobel Prize in 1908 (Vasiljevic and Shah, 2008). Probiotics are live microorganisms that benefit the health of the host, if consumed in adequate amount (Liaskovskii and Podgorskii, 2005). Mostly probiotic bacteria are incorporated in dairy products like milk, ice cream and yoghurt. The lactic acid producing probiotics keep the gut at a low pH, maintains the gut microflora and helps preserve the dairy products.

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They also combat the growth of harmful pathogens that cause foodborne illnesses (that is, diarrhea). The probiotics prevent the attachment of these pathogens by competing for similar binding sites on the gut epithelium (Parvez et al., 2006). But to deliver the health benefits, the probiotic bacteria need to be viable in the gut. The International Dairy Foundation (IDF) has recommended a minimum number of $10^7$ CFU per gram of the product consumed (Homayouni et al., 2008). However, the loss of viability can happen prior to consumption, during processing procedures such as oxygen stress, freezing and drying or due to the harsh conditions in gastrointestinal (GI) tract such as high pH, bile salt and gastric acid secretion (Chávarri et al., 2010).

Therefore, providing a physical barrier to the probiotic living cells to resist adverse environmental conditions is therefore an approach currently receiving considerable interest (Kailasapathy, 2009).

The microencapsulation techniques have resulted in greatly enhanced viability of these microorganisms in food products as well as in the gastrointestinal tract. In this process, active agents are entrapped within a carrier material. It is a useful tool to improve living cells into foods, to protect (Favaro-Trindade and Grosso, 2002; Liserre et al., 2007; Shima et al., 2009; Thantsha et al., 2009) and to extend their storage life and to convert them into a powder form for convenient use (O’riordan et al., 2001; Lian et al., 2003; Oliveira et al., 2007). In addition, microencapsulation can promote controlled release and optimize delivery to the site of action, thereby potentiating the efficacy of the respective probiotic strain. This process can also prevent these microorganisms from multiplying in food that would otherwise change their sensory characteristics.

The investigations has revealed that the probiotic cell adhesion on the gut lining is considered as an important requirement for delivering health based benefits and the estimation of surface hydrophobicity is the best technique to determine this adherence and colonization potential (Kaushik et al., 2009).

In this study, two materials of particular interest used as capsules were the Ca-alginate and prebiotics amalgum (containing galactooligosaccharides (GOS), mannooligosaccharide (MOS), and fructooligosaccharides (FOS)). Alginate is a natural polysaccharide of β-D-mannuronic acid (M) and α-L-guluronic acid (G), usually extracted from algae/seaweed and is currently the most widely used and studied material of microencapsulation (Chen and Walker, 2005). While Prebiotics are a group of carbohydrates made up of functional oligosaccharides which are non-digestible food substances that favour the growth of other bacteria (Liaisovskii and Podgorskii, 2005).

The main objective of this research was to evaluate the adherence potential and survival of indigenous probiotic bacteria in gastrointestinal (GI) environment after microencapsulation.

**MATERIALS AND METHODS**

**In vitro adherence studies of indigenous probiotic strains**

**Cell surface hydrophobicity**

To determine the probiotics adhesion to hydrocarbons the method of Rosenberg et al. (Rosenberg et al. 1980) and absorbance was taken at 600 nm and surface hydrophobicity (%) was calculated in replicates as percent decrease ($\Delta A_{abs} \times 100$) in the absorbance of suspension ($A_{inm}$) and after phase separations ($A_{final}$) as follows:

$$Surface \ Hydrophobicity \ (%) = \frac{A_{initial} - A_{final} (\Delta A)}{A_{initial}} \times 100$$

**Cell aggregation**

The freshly grown probiotic bacterial cells in MRS broth at 35°C for 24 h were harvested (1) and the cell pellet washed with PBS and resuspended in PBS. An absorbance of ~0.5 at 600 nm ($A_{initial}$) was taken. Then the suspension was centrifuged and the pellet was resuspended in equal volume of removed broth (in 1). The mixture was kept at 35°C for 2 h after that 1.0 ml of the top suspension was taken to measure the absorbance ($A_{final}$), and broth was used as reference (Del Re et al., 2000; Tomás et al., 2005). The estimation was performed in replicate. To calculate cellular autoaggregation index, the difference in percentage between the initial and final absorbance was recorded as follows:

$$Aggregation \ (%) = \frac{A_{initial} - A_{final} (\Delta A)}{A_{initial}} \times 100$$

**Caco-2 Cells Adhesion Assay**

Adhesion of probiotic isolates was estimated using method of Jacobsen et al. (Jacobsen et al., 1999). with Caco-2 cells ($10^5$) and the estimation was performed in replicate.

**Preparation of cell suspension for microencapsulation**

All the previously isolated Probiotic cultures (Hassan and Chaudhry, 2013) were prepared, from frozen stocks stored at −80°C, by transferring into MRS broth, then cultures were incubated,
in anaerobic conditions, at 35°C for 18 h. After incubation, media were centrifuged for 10 min at 4°C, and cells were washed with sterile 0.1% (w/v) pepton water.

**Microencapsulation**

For microencapsulation, firstly the cells were coated with Ca-alginate by the emulsion method of Ortakci (Ortakci et al., 2012) and stored in pepton-saline solution at 4°C until use. Later, the alginate-encapsulated probiotic cells were coated with the mixture of prebiotics, containing FOS, MOS, GOS and free cells were used as control. The overall adopted technique for microencapsulation is shown in Figure 1.

**Survival evaluation in simulated gastric juice (SGJ)**

To investigate the effect of pH, same as that of Gastric juice, on the survival of indigenous encapsulated probiotic bacteria, the cells were treated (for 120 min) with sterile stimulated Gastric Juice -A (SGJ -A) as followed by Mainville et al. (Mainville et al., 2005) containing 2.0 g/kg of NaCl and 0.3 g/kg of pepsin in M HCl (pH 1.4) and sterile stimulated Gastric Juice- B (SGJ -B) containing 0.9 M H₃PO₄ (pH 2.0) instead of HCl.

**Survival evaluation in simulated intestinal juice (SIJ)**

After treatment in SGJ for 60 min, the mixture was converted to simulated intestinal juice (Huang and Adams 2004; Annan et al. 2008) by adding to 36 mL of the pancreatin-bile mixture (containing 1 mg/mL pancreatin and 4.5 g/mL of bile salts in phosphate buffer), and pH was adjusted to 7.4. The mixture was then incubated for 4 h at 35°C.

**Statistical analysis**

The data observed in in vitro adherence studies as well as the logarithmic reductions in free (control), single encapsulated and double encapsulated bacterial cells as a consequence of acidic (SGJ-A and SGJ-B) and bile (SIJ) juices were analyzed by one-way ANOVA using SPSS 17 version. Significance was declared at $P \leq 0.05$.

**RESULTS AND DISCUSSION**

**In vitro adherence studies of potential indigenous probiotic strains**

**Cell surface hydrophobicity**

The investigations has revealed that the probiotic cell adhesion on the gut lining is considered as an important requirement for delivering health based benefits and the estimation of surface hydrophobicity is the best technique to determine this adherence and colonization potential (Kaushik et al., 2009). Therefore, the indigenous probiotic
isolates were also analysed for the adherence studies in this study. The mean with standard deviation of triplicate experiments are presented in Table 1. It has been found that there was no significant difference in the hydrophobicity of both solvents used. All the recorded readings found in line with Kaushik et al. (Kaushik et al., 2009). In the present estimation, the hydrophobicity was found with mixed trend, the indigenous Lactobacillus acidophilus was found with highest cell surface hydrophobicity (56.3%) and lowest was found in Lactobacillus reuteri (28.1%). Previous research has revealed that some lactobacillus strains show low cell surface hydrophobicity to 2-5% (Schillinger et al., 2005; Rijnaarts et al., 1993) but neither of the isolated strain showed such a low hydrophobicity percentage. Kaushik et al. (2009) has claimed that hydrophobicity plays a vital role in the cellular interaction. This great difference in the hydrophobicity percentage could be due to the difference in the cell surface protein expression that may be due to the variation in environmental conditions (Tomás et al., 2005; Ramiah et al., 2007).

### Cell auto-aggregation

The reports have highlighted the presence of four mucus-binding proteins genome in Lactobacillus plantarum including the longest gene, ORF lp_1643 containing six repeats. These proteins helps in the adherence and the persistence of probiotic bacteria in the host GI tract (Boekhorst et al., 2006b) (Boekhorst et al., 2006a). It has also been investigated that after adherence in the gut lining, the probiotic bacteria got the ability of aggregation and colonization for health promoting benefits. Vandevooorde et al. (1992) has also reported that the cellular aggregation not only facilitates the colonization but also provide a protection to the host by biofilm formation (Vandevooorde et al., 1992) and this biofilm formation is important for functioning of probiotics (Ocaña and Nader-Macias, 2001; Kos et al., 2003; Cesena et al., 2001). In the present study, the mean and standard deviation of percentages of triplicate experiment for cell aggregation of all indigenous isolates were recorded and presented in Table 1. The highest percentage was observed in L. rhamnosus (51.9%) followed by L. brevis with the percentage of 56.1 while the least was found in two isolates L. casei and L. fermentum with the similar value of 31.7%. The cell aggregation study was also concluded by Kaushik et al (Kaushik et al., 2009) but they did not find such a high potential of self-aggregation in their indigenous strain of Lactobacillus plantarum.

### Cell adhesion assay

The mean and standard deviation of cell adhesion assay of selected indigenous isolates are given in Table 1, the highest percentage was observed by L. casei (8.6%)

---

**Table 1.** Cell surface hydrophobicity, aggregation, and cell adhesion evaluation of selected indigenous probiotic isolates.

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Hydrophobicity (%)</th>
<th>Aggregation (%)</th>
<th>Adhesion ratio (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n-hexadecane</td>
<td>Xylene</td>
<td></td>
</tr>
<tr>
<td>L. acidophilus</td>
<td>56.3 ± 0.10</td>
<td>56.1 ± 0.04</td>
<td>36.1 ± 0.13</td>
</tr>
<tr>
<td>L. lactis</td>
<td>49.1 ± 0.06</td>
<td>49.3 ± 0.08</td>
<td>34.9 ± 0.14</td>
</tr>
<tr>
<td>L. casei</td>
<td>52.7 ± 0.21</td>
<td>52.3 ± 0.01</td>
<td>31.7 ± 0.09</td>
</tr>
<tr>
<td>L. rhamnosus</td>
<td>47.3 ± 0.14</td>
<td>47.3 ± 0.09</td>
<td>41.3 ± 0.98</td>
</tr>
<tr>
<td>L. brevis</td>
<td>43.1 ± 0.12</td>
<td>43.5 ± 0.03</td>
<td>32.6 ± 0.01</td>
</tr>
<tr>
<td>L. lactis (b)</td>
<td>37.5 ± 0.19</td>
<td>37.6 ± 0.13</td>
<td>40.9 ± 0.12</td>
</tr>
<tr>
<td>L. rhamnosus (b)</td>
<td>39.1 ± 0.24</td>
<td>39.1 ± 0.17</td>
<td>51.9 ± 0.07</td>
</tr>
<tr>
<td>L. acidophilus (b)</td>
<td>34.9 ± 0.14</td>
<td>34.1 ± 0.74</td>
<td>37.9 ± 0.07</td>
</tr>
<tr>
<td>L. brevis (b)</td>
<td>33.4 ± 0.14</td>
<td>33.6 ± 0.79</td>
<td>56.1 ± 0.03</td>
</tr>
<tr>
<td>L. casei (b)</td>
<td>36.7 ± 0.18</td>
<td>36.2 ± 0.18</td>
<td>48.0 ± 0.04</td>
</tr>
<tr>
<td>B. bifidus</td>
<td>29.1 ± 0.21</td>
<td>29 ± 0.06</td>
<td>49.2 ± 0.08</td>
</tr>
<tr>
<td>B. infantis</td>
<td>39.7 ± 0.17</td>
<td>39.1 ± 0.08</td>
<td>32.9 ± 0.06</td>
</tr>
<tr>
<td>B. longum</td>
<td>51.3 ± 0.78</td>
<td>51.6 ± 0.07</td>
<td>32.5 ± 0.02</td>
</tr>
<tr>
<td>B. dentum</td>
<td>46.7 ± 0.98</td>
<td>46.7 ± 0.17</td>
<td>33.3 ± 0.07</td>
</tr>
<tr>
<td>B. brevis</td>
<td>31.5 ± 0.15</td>
<td>31.3 ± 0.04</td>
<td>31.8 ± 0.03</td>
</tr>
<tr>
<td>B. adole</td>
<td>38.4 ± 0.18</td>
<td>38.1 ± 0.09</td>
<td>40.7 ± 0.07</td>
</tr>
<tr>
<td>L. plantum</td>
<td>36.6 ± 0.24</td>
<td>36.1 ± 0.13</td>
<td>49.6 ± 0.05</td>
</tr>
<tr>
<td>L. reteri</td>
<td>28.1 ± 0.27</td>
<td>28.6 ± 0.19</td>
<td>31.9 ± 0.03</td>
</tr>
<tr>
<td>L. gasser</td>
<td>35.5 ± 0.17</td>
<td>35.5 ± 0.19</td>
<td>35.1 ± 0.10</td>
</tr>
<tr>
<td>L. fermentum</td>
<td>39.6 ± 0.12</td>
<td>39.6 ± 0.27</td>
<td>31.7 ± 0.09</td>
</tr>
</tbody>
</table>
followed by isolate L. brevis with the percentage of 8.5% while the least was found in isolates Lactobacillus lactis with the value of 6.1%. The results of cell surface hydrophobicity, cell auto-aggregation and cell adhesion assay for the selected indigenous probiotic isolates suggests that these isolates have specific interaction and colonization potential in the gut that is also indicated by good adhesion ratio while using Caco-2 cell lines, as matches the conclusions of Kaushik on lactobacillus strains (Kaushik et al., 2009).

This study showed significant effects on the survival of free cultures in both SGJ-A and SGJ-B while the loss was higher in single microencapsulated L. plantarum and Lactobacillus paracasei while negligible loss was observed for double- microencapsulated cells. The zero loss was found in the mean probiotic count (cfu/g) of Lactobacillus delbrueckii subsp. Bulgaricus and L. paracasei. While a slight revival was observed in the free and single encapsulated bacteria in SIJ probably because of resuscitation of some injured cells because of acidic conditions. The adherence studies reveal that there is significant potential of health benefits of the isolated indigenous probiotic cultures and the combination of Ca-alginate and prebiotics significantly improves the viability of probiotic cells in the harsh gastrointestinal conditions.

These microencapsulated indigenous probiotic cultures can be used in food technology and production as it provides a solution to the low viability of probiotics incorporated in local dairy products. Ideally these cultures, since have adherence potential, can maintain the level of the beneficial probiotic bacteria at the minimum standard amount required (Akhiar and Aqilah, 2010).

### Microencapsulation and survival studies of indigenous probiotic strains

The mean and standard deviation experiments conducted in triplicate for the free, single (Ca-alginate) and double (Ca-alginate and prebiotics amagum) microencapsulation are shown in Table 2 and count was performed on seven day interval for the period of six weeks. The rate of reduction of probiotic bacterial count (CFU/g) was calculated by the following formula;

\[
Rate\ of\ Reduction\ % = \left( \frac{\text{Viable cell count (CFU/g)}}{\text{Initial Viable count at zero hour storage (CFU/g)}} \right) \times 100
\]

All the individual cultures of Lactobacillus and Bifidobacterium as well as the mixed culture of both showed similar response as that was investigated by Sultana et al. (2000), Kailasapathy et al. (2002), Vivek et al. (2013) and Chen et al. (2005), and proved the potential of microencapsulation for protecting the indigenous probiotic bacterial cells. The adopted double encapsulation technique has presented a significant effect (P < 0.05) on the survival of probiotic indigenous cells during storage of six weeks. In the case of free cells, which were used as a control, the reduction in the mean count was observed on day 28, single encapsulated on day 35 while the double encapsulated bacterial cells was decreased from 3.84 × 10^9 on day 35 to 3.83 × 10^9 on day 42.

The present investigation also endorsed the results of Yeo and Liong that a significant increase in the count for L. acidophilus FTDC 8033 and Lactobacillus sp. FTDC 2113 in soy milk when supplemented with prebiotics (FOS) upto 7-log CFU/ml (Yeo and Liong, 2010). According to Ding and Shah, probiotic bacteria encapsulated in alginate, xanthan gum, and carrageenan gum survived better (P < 0.05) than free probiotic bacteria, in acidic environment (Ding and Shah, 2009), which also justifies the significance of present study. In fact, it has been investigated that prebiotics (fructooligosaccharides, iso-maltooligo- saccharides and lactulose) is an emerging alternative that can further enhance probiotic activity and they enhance the growth of probiotics by providing carbon and nitrogen.

### Table 2. Probiotic bacterial count for Free, Single and Double Encapsulated bacteria during storage.

<table>
<thead>
<tr>
<th>Time (day)</th>
<th>Control (Mean CFU × 10^6/g ±SD)</th>
<th>Single-encapsulated (Mean CFU × 10^6/g ±SD)</th>
<th>Double-encapsulated (Mean CFU × 10^6/g ±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>2.45 ± 0.20</td>
<td>2.98 ± 0.21</td>
<td>3.16 ± 0.18</td>
</tr>
<tr>
<td>7</td>
<td>2.52 ± 0.14</td>
<td>2.92 ± 0.18</td>
<td>3.32 ± 0.09</td>
</tr>
<tr>
<td>14</td>
<td>2.68 ± 0.19</td>
<td>3.12 ± 0.23</td>
<td>3.41 ± 0.17</td>
</tr>
<tr>
<td>21</td>
<td>2.74 ± 0.27</td>
<td>3.29 ± 0.40</td>
<td>3.68 ± 0.23</td>
</tr>
<tr>
<td>28</td>
<td>2.63 ± 0.23</td>
<td>3.36 ± 0.23</td>
<td>3.73 ± 0.71</td>
</tr>
<tr>
<td>35</td>
<td>1.91 ± 0.20</td>
<td>3.29 ± 0.16</td>
<td>3.84 ± 0.20</td>
</tr>
<tr>
<td>42</td>
<td>0.71 ± 0.19</td>
<td>3.14 ± 0.14</td>
<td>3.83 ± 0.20</td>
</tr>
</tbody>
</table>
sources which can increase their colonization of the gut (Annan et al., 2008).

However, the symbiotic effects of probiotics and prebiotics are strain-dependent. As Yeo Siok had reported different growth behaviours for six different strains of lactobacilli and bifidobacteria when supplemented with five different prebiotics. Lactobacillus spp. FTDC 2113 grew significantly when supplemented with FOS but did not grow with pectin (Yeo and Liong, 2010). In the present study the mixture of encapsulated bacteria has showed higher growth in the presence of prebiotics. The survival of double microencapsulated probiotics not only proves the statement of Rabanel et al. that both the alginate and prebiotics are compatible with probiotics (Rabanel et al., 2009) but also justifies the claim of Chen Kun-Nan et al. (2005) that the Survival rate of the co-encapsulated active probiotics increases 1000 times higher than for alginates alone.

Simulated gastric Juice

The stimulated Gastric Juice (SGJ-A) has shown a significant reduction on the free probiotic cell (Figure 2). Initial mean count for free cells was 2.45 × 10^8 cfu/g which had lost 1.09 × 10^7, 1.04 × 10^7, 2.01 × 10^7, 2.74 × 10^7, 2.56 × 10^7, 1.76×10^7 and 1.99 × 10^7 for L. lactis AH-1, L. reuteri AH-2, L. acidophilus AH-3, Lactobacillus rhamnosus AH-4, Lactobacillus casei AH-5, L. plantarum AH-6, and Lactobacillus brevis AH-7, respectively.

The results of the survival evaluation of free cells, Single encapsulated and double encapsulated probiotics in stimulated Gastric Juice B, shown in Figure 3. The results indicated that the pH 2.0 resulted in great loss in count for uncoated free bacterial cells while the coated bacterial cells, either single or double microencapsulated, had a negligible reduction.

In the case of single microencapsulated probiotics, the loss was high L. plantarum AH-6 and Lactobacillus brevis AH-7 that is, 2.29 × 10^5 and 3.24 × 10^5, respectively. The bacterial loss was negligible in the case of double- microencapsulated bacterial cells. The zero loss was found in the mean microbiological count (cfu/g) of L. reuteri AH-2 and L. reuteri AH-7 after the incubation of 2 h in SGJ-A. The results of bifidobacterium cultures are shown in Figure 4 for SGJ-A.

This rapid reduction in count of free bacterial cells, when incubated for 120 min in stimulated Gastric Juice -A (SGJ-A), was the function of low pH (1.4) and acidic conditions, which is similar to the pH of the human stomach before ingestion of food. the main reason of rapid loss was that no protection was provided to free cells in such a harsh acidic environment. The technique of double microencapsulation, both with Ca- alginate and prebiotics (FOS and IMO) showed amazing results in the case of L. reuteri AH-2 and Lactobacillus brevis AH-7 as compared to the negligible loss of bacterial count was observed in the case of other probiotic isolates. It proves the potential in technique of double microencapsulation. Under the double microencapsulated probiotic cells, the survival of bacteria was significantly greater than free and single-microencapsulated bacterial cells. This supports the notion that it is prime to test the probiotics in the proper physiological conditions as bacterial survival is greatly influenced by variations in pH (Mainville et al., 2005; Pitino et al., 2010). This agrees with the findings of Sharp (Sharp et al., 2008) who observed a 3.8-log reduction after two hours of incubation in SGJ.
Figure 3. Mean loss in Lactobacillus count (Log 10 cfu/g) for free (control), Single encapsulated and Double Encapsulated bacteria in SGJ-B. *L. lactis* AH-1, *Lactobacillus reuteri* AH-2, *Lactobacillus acidophilus* AH-3, *Lactobacillus rhamnosus* AH-4, *Lactobacillus casei* AH-5, *Lactobacillus plantarum* AH-6, and *Lactobacillus brevis* AH-7.

Figure 4. Mean loss in bifidobacterium and two lactobacillus strains count (Log 10 cfu/g) for free (control), Single encapsulated and Double Encapsulated bacteria in stimulated Gastric Juice B (120 min incubation).

Again the great loss of free probiotic cells was found in the SGJ-B as compared to single and double - micro-encapsulation. The *in vitro* test of gastric survival using H$_3$PO$_4$ (SGJ-B) was useful to provide a buffering effect when the bacteria were present in a uncoated form especially. The results of SGJ-B for the bifidobacterium isolates
Mean loss in bifidobacterium count
In Stimulated Gastric Juice-B

![Graph showing mean loss in bifidobacterium count.](image)

**Figure 5.** Mean loss in bifidobacterium and two lactobacillus strains count (Log 10 cfu/g) for free (control), Single encapsulated and Double Encapsulated bacteria in stimulated Gastric Juice B (120 min incubation).

Mean loss in lactobacillus isolates (Log 10 cfu/g) in Stimulated Intestinal Juice

![Graph showing mean loss in lactobacillus isolates.](image)

**Figure 6.** Mean loss in Lactobacillus isolates (Log 10 cfu/g) for free (control), Single encapsulated and Double Encapsulated bacteria in SIJ. *L. lactis* AH-1, *Lactobacillus reuteri* AH-2, *Lactobacillus acidophilus* AH-3, *Lactobacillus rhamnosus* AH-4, *Lactobacillus casei* AH-5, *Lactobacillus plantarum* AH-6 and *Lactobacillus brevis* AH-7.

are shown in Figure 5.

**Simulated intestinal juice (SIJ)**

The Mean loss in probiotic count (Log 10 cfu/g) for control, single encapsulated and double encapsulated probiotics in stimulated Intestinal Juice (pH 7.4) are shown in Figure 6. A slight recovery of free and single encapsulated bacterial strains was found after the addition of bile-pancreatin mixture. The acids of the SGJ-A and SGJ-B both were unable to provide any significant protective effect for the control and single micro-encapsulated probiotics. A slight increase may be the resuscitation of some cells that were sublethally injured during the 160 min of incubation of SGJ, while the zero loss was found in the case of double microencapsulated bacterial cells.

*Bifidobacterium breve* and *Bifidobacterium longum* were encapsulated by Picot and Lacroix, who found
results similar to the present study. They reported that this approach is potentially useful for delivery of viable probiotics to the gastrointestinal tract of humans (Picot and Lacroix 2004). After incubation in simulated gastric (1 h) and intestinal juices (pH 7.4, 4 h), the number of probiotic *B. adolescentis* the surviving cells was found high (Annan et al. 2008) showing similarity with the present study. The results of SIJ for the bifidobacterium and two lactobacillus isolates are shown in Figure 7.

This study showed significantly adverse effects on the survival of free cultures in both SGJ-A and SGJ-B while the loss was higher in single microencapsulated *L. plantarum AH-6* and *Lactobacillus brevis AH-7* while negligible for double- microencapsulated cells. The zero loss was found in the mean microbiological count (cfu/g) of *L. reuteri AH-2* and *Lactobacillus brevis AH-7*. While a slight revival was observed in the free and single encapsulated bacteria in SIJ probably because of resuscitation of some injured cells because of acidic conditions. The findings reveal that the combination of Ca-alginate and prebiotics significantly improve the viability of bacterial cells in the harsh gastrointestinal conditions and may be of use for the food and/or pharmaceutical industries. In the nutshell, the results of survival studies reveals that incorporation of ca- alginate and prebiotic amalgam has improved viability of probiotics.

**REFERENCES**

Akhiria, M., Aqilah NS (2010). Enhancement of probiotics survival by microencapsulation with alginate and prebiotics. MMG 445 Basic Biotechnol. 6 (1)


Favaro-Trindade C, Grosso C (2002) Microencapsulation of *L. acidophilus* (La-05) and *B. lactis* (Bb-12) and evaluation of their survival at the pH values of the stomach and in bile. Microencaps 19(4):485-494.


**Figure 7.** Mean loss in bifidobacterium and two lactobacillus strains count Log 10 cfu/g) for free (control), Single encapsulated and Double Encapsulated bacteria in SIJ.


