Co-encapsulation of *Lactobacillus acidophilus* with Jerusalem artichoke in alginate-chitosan matrix

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**ABSTRACT:** The main problem of probiotics is the low survival of these microorganisms in food products and sensitive to harsh conditions during food processing, storage or gastrointestinal tract. The suitable technology for protect probiotics to remain the number of probiotic cells in food product and also survive within the host, is microencapsulation technique. The aim of this work was to evaluate the survivability of co-encapsulated probiotics *Lactobacillus acidophilus* TISTR 1338 with complementary prebiotics (Jerusalem artichoke and inulin*). Two prebiotics were tested by in vitro fermentation which were separately encapsulated within alginate matrix and coat with chitosan. Their efficacy in improving the viability of probiotic bacteria was evaluated against sequential stress environments of freeze-drying at -46 °C and high temperature condition which exposed to 70 °C for 30 min as heat generated in feed pelleting process. The results revealed that the survival rate of co-encapsulated cells with Jerusalem artichoke was 77.3% which significantly differences higher than the co-encapsulated cells with inulin* and control (encapsulate without prebiotic) after heat processing. In part of freeze-drying process, the survival rate of co-encapsulated cells with Jerusalem artichoke was 85.72% and co-encapsulated cells with inulin* was 82.74% there are no significant differences. Therefore, co-encapsulation probiotics with Jerusalem artichoke could be a useful to protect the cells from freeze drying condition and high temperature in food and feed processing.

**Keywords:** microencapsulation, probiotic, Jerusalem artichoke, inulin

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**Introduction**

Nowadays, probiotics and prebiotics have been widely accepted as a natural means to promote health for both humans and animals. They are used as health supplements in food and feeds to replace the use of antibiotics or chemical supplements which are chemical compound. This has led to widespread problems of drug resistance and unacceptance from consumers. There is the need to look for viable alternatives to modulate gastrointestinal health and reduce the massive use of antibiotics; consequently, probiotics and prebiotics are natural strategies to defense mechanisms of human or animal health. Probiotic bacteria are defined as “live microorganisms which, when administered in adequate amounts, confer a health benefit on the host” (FAO/WHO, 2002). Probiotic beneficially affect host’s health by improving the gut microbiota balance and the defenses against pathogens, including stimulation of the immune system, blood cholesterol reduction, vitamin synthesis, anti-carcinogenesis and anti-bacterial activities (Sarao and Arora, 2015). In the
meantime, the use of probiotic bacteria in feed additives has become increasingly popular for improved nutrition, healthy digestion, and disease prevention. Whereas prebiotics are defined as non-digestible food ingredients that selectively stimulate the growth and/or the metabolism of health-promoting bacteria in the intestinal tract, thus this improves an organism’s intestinal balances of the organism (Gibson and Roberfroid, 1995). It has been suggested that adding prebiotics to certain foods may increase the viability of probiotic bacteria passing through gastrointestinal tract and thus exert a beneficial effect on host’s health. (Fooks et al., 1999; Iyer and Kailasapathy, 2005; Roberfroid, 2000; Khalf et al., 2010). Commonly prebiotic oligosaccharides are used as consumer products such as inulin, fructooligosaccharides (FOS), mannoooligosaccharides (MOS) and galactooligosaccharides (GOS). The combination of probiotics and prebiotics are referred to as synbiotics (Gibson and Roberfroid, 1995) which is growing interest to develop in food industry, feed supplement and pharmaceuticals. However, the maintenance of the cell viability in probiotic-containing products is still considerably challenging because the probiotic must survive during the industrial processing, storage condition and gastrointestinal passage. The suitable technology for protection of probiotic cells has resulted in greatly enhanced viability of these microorganisms in food products as well as in the gastrointestinal tract, is microencapsulation technique. Microencapsulation is a process to entrap probiotic cells within a carrier material and it is a useful tool to improve living probiotic cells into foods, to protect them from their external environment and to extend their storage life (Capela et al., 2007; Chávarri et al., 2012). The most widely used matrix for microencapsulation is alginate which has been found to increase the survival of probiotics from 80 to 95% (Mandal et al., 2006). In addition, research is ongoing to develop microcapsule for a better resistance to stress environment by double or triple layers of alginate-chitosan matrix. *Lactobacillus acidophilus* 547 and *Lactobacillus casei* 01 were encapsulated in alginate beads coated with chitosan has been reported to improve the survival of probiotics in both yogurt and severe conditions, such as in simulated gastric, intestinal juices and bile salt solution (Krasaekoopt et al., 2004). Nualkaekul et al. (2012) also demonstrated that microcapsulated *Lactobacillus plantarum* in double chitosan coat was improved the survivability of probiotic cells in pomegranate juice. Moreover, several studies showed that the combine both prebiotics and alginate coating materials may better protect probiotic in food systems and the gastrointestinal tract due to synbiosis (Chen et al., 2005; Nazzaroa et al., 2009; Okuro et al., 2013; Krasaekoopt et al., 2014). Some studies have shown that probiotics strain remain viable for prolonged periods in yogurts or refrigerated milk when co-encapsulated with prebiotic like inulin (Desai et al., 2004; Capela et al., 2006). GOS incorporated into microcapsules increase the survival of *L. acidophilus* and *L. casei* in orange juice under refrigerated storage (Krasaekoopt et al., 2014). The most investigated prebiotic substances are fructan-based inulins and oligofructoses (Roberfroid et al., 1998). Among other plants rich in inulin and FOS is Jerusalem artichoke. Kaentawan or Jerusalem artichoke (JA) (*Helianthus tuberosus*) is a tuberous annual crop of which tubers are rich
in fructooligosaccharide carbohydrates in the forms of inulin and fructans. The FOS are not digestible by the animal digestive enzymes but are readily digested by the beneficial microbes in gastrointestinal tract (Prosky, 1999; Patkai et al., 2002). Conventionally, Jerusalem artichoke has been used for food or animal feed (Swanton and Hamill, 1994). Recently, alternative uses have been explored to produce functional food ingredients such as inulin, oligofructose and fructose (Panchev et al., 2011). However, there are less research about Jerusalem artichoke on the synbiotic application. Therefore, the aim of this work was to investigate the effect of Jerusalem artichoke and inulin® on the survival of encapsulated Lactobacillus acidophilus TISTR 1338 within alginate matrix and coat with chitosan after freeze-drying process and heat processing.

Materials and Methods

Probiotics and prebiotics preparations

Pure cultures of probiotic bacteria L. acidophilus TISTR 1338 was obtained from Thailand Institute of Scientific and Technological Research. The culture was transferred twice in MRS broth and incubated at 37 °C for 24 hrs under anaerobic condition. Cells were harvested by centrifugation at 3000×g for 15 min at 4 °C and the cell pellet was washed twice with sterile saline solution. The cells were then either directly used in the assay or subjected to microencapsulation.

Inulin® was obtained from Nutrition SC Company, Thailand. The tubers Jerusalem artichoke was purchased from Piriya farm (Nakhon Ratchasima Province, Thailand). The tubers were washed, peeled, sliced, dried in hot air oven for 24 hrs and grinded to powder and stored at 4 °C.

Selection of complementary prebiotics by in vitro fermentation

A minimal medium free of carbon source was used to investigate the ability of L. acidophilus TISTR 1338 to grow on different prebiotic, inulin® and Jerusalem artichoke, and also in carbon-free medium, which acted as control. The minimal growth medium (pH 6.8) contained 1.0% beef extract, 0.3% yeast extract, 1.0% pancreatic digest of casein, and 0.5% NaCl. The prebiotics were added aseptically to the medium at 2.0% (w/v). Criteria for the evaluation of the use of prebiotics were the growth of Lactobacillus and acidification compared with the results obtained in the control medium. Anaerobic fermentation was carried out in a 250-mL shake flask and incubated at 37 °C for 48 hrs. Growth was measured by serial dilutions method and viable numbers enumerated using spread plating on MRS agar, and colonies were counted after 48 hrs of anaerobic incubation at 37 °C. The acidification by these strains was determined as changes in pH using a pH meter (modified Iyer and Kailasapathy, 2005).

Microencapsulation

The beads were prepared by the extrusion method according to the method described by Trabelsi et al. (2013) with some modification. Briefly prebiotic solution, 3% (w/v) sodium alginate, 5.5% (w/v) MRS broth, and 3% (w/v) inulin® or Jerusalem artichoke were mixed in distilled water and sterilized by autoclaving at 121 °C for 15 min. The cells suspension was mixed in prebiotic solution and then injected through a 30G
needle into sterile 0.1 M CaCl$_2$ solution. The syringe was hold 5 cm above CaCl$_2$ solution. The droplets immediately formed in gel spheres. The microcapsules were allowed to harden for 30 min in CaCl$_2$ and then washed twice with 0.1% peptone solution to remove excess calcium ions and untrapped cells. The microcapsules were then separated by using filter paper and then further coat with chitosan. Chitosan solution was prepared by dissolving 8 g of chitosan in 950 mL of 0.1 M acetic acid. The pH was adjusted to 5.7 - 6.0 with 1 M NaOH. The solution was filtered through a Whatman No.4 filter paper and adjusted to 1 L by distilled water and autoclaved at 121 °C for 15 min. The microcapsules were transferred to 0.8% (w/v) chitosan solution and stirred gently with a magnetic bar for 60 min to ensure the evenly coated of the surface of the microcapsules. Such microcapsules were then separated by using a sieve and rinsed twice with distilled water and then frozen at -24 °C for 12 hrs. The final microcapsules were obtained using a vacuum freeze-dryer at -46 °C for 36 hrs. After that, freeze-dried microcapsules were stored in closed containers at 4 °C until use.

**Bacterial enumeration**

To determine the viable cells of the microencapsulated *L. acidophilus* TISTR 1338, 1 g of microcapsule was re-suspended in 10 ml phosphate buffer (0.1 M, pH 7.4), gently shaken for 30 min at room temperature. Samples were taken at different time intervals to determine the complete release of encapsulated bacteria by spread plating on MRS agar. The plates were incubated at 37 °C for 48 hrs. Sterile sodium chloride solution 0.85% (w/v) was used to prepare the serial dilutions. Free bacteria were also enumerated on MRS agar using the same technique.

**Determination of encapsulation efficiency**

The encapsulation efficiency (EE), the efficiency of entrapment and survival of viable cells during the microencapsulation process, was calculated by the following expression;

$$EE(\%) = \frac{X_t}{X_i} \times 100$$  \hspace{2cm} (1)

Where $X_t$ is the total amount of probiotic loaded in microcapsules and $X_i$ is the initial amount of probiotic added in the preparation process.

**Survival rate of microencapsulated cells after freeze-drying process and heat processing**

Survival rate of the viable cells of the *L. acidophilus* TISTR 1338 after freeze-dried and heat processing in microcapsules was calculated by the following expression;

$$Survival\ rate(\%) = \frac{F_a}{F_b} \times 100$$  \hspace{2cm} (2)

Where $F_a$ is the amounts of viable cells in microcapsules after freeze-drying and $F_b$ is the amounts of viable cells in microcapsules before freeze-drying process.

**Viability of microencapsulated cells under heat processing**

The free and encapsulated *L. acidophilus* TISTR 1338 were assayed for heat tolerance at 70 °C for 30 min. Freeze-dried microcapsules 0.1 g were transferred into feed machine and dry in hot air oven. Survivals encapsulated cells were enumerated by plating on MRS agar as described previously.
Statistical analysis

All experiments and analyses are presented as mean ± standard deviation in triplicate trial. The data were subjected to one-way analysis of variance (ANOVA) and the significance of the difference between means was determined by Duncan’s multiple range test (P<0.05) using SPSS version 21 for windows.

Results and Discussion

Selection of complementary prebiotics

Results of the selection complementary prebiotic showed that there was no significant difference among the prebiotics tested between inulin® and JA tuber powder on bacterial growth _L.acidophilus_ TISTR 1338 were grown in prebiotics indicated that these strains could use the prebiotics as a carbon source to sustain their growth as shown in Figure 1. There was no significant difference among the prebiotics tested on the amount of cell growth while these strains grew very poorly on the control treatment with no carbon source, thus, this might confirm that the base medium was carbon limiting to the growth of this strain. As the same result in the acidification by _L. acidophilus_ TISTR 1338, the acidification of the strain in these two prebiotics were not significantly different whereas prebiotic treatment was significantly better compared with control treatment (Table 1). Iyer and Kailasapathy (2005) was observed for a number of _L. acidophilus_ strains and suggested that these organisms were able to grow well on oligosaccharides composed predominantly of fructose moieties than the monosaccharide fructose. Biedrzycka and Bielecka (2004) reported that the in vitro consumption of inulin by bifidobacteria depended on purity and DP of FOS chains. Sathyabama _et al._(2014) revealed that the variety of oligosaccharide composition could be the reason for difference in bacterial growth rate. In this part, the results show that JA tuber can be potentially used as a complementary prebiotic for the _L. acidophilus_ TISTR 1338. Owing to composition of JA which is rich in FOS carbohydrates in the forms of inulin and fructans. Elaheh _et al._ (2016) indicated that the degree of polymerization of fructans was an important factor that provided the accessibility of fructans to the bacteria.
Table 1. Acidification of *L. acidophilus* TISTR 1338 in minimal media (after 48 hrs of fermentation) containing different prebiotic as carbon sources.

<table>
<thead>
<tr>
<th>Prebiotic</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (no carbon source)</td>
<td>6.70±0.03&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Inulin&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.90±0.14&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Jerusalem artichoke</td>
<td>5.10±0.16&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a,b</sup> Means±standard deviation with different superscript in the same column are significantly different (P<0.05) according to DMRT.

Figure 1. Selection of complementary prebiotics by fermentation of *L. acidophilus* TISTR 1338. Growth was measured as log CFU/mL. The error bars represent standard deviation of means (n=3).

Number of cells entrapped and encapsulation efficiency of microcapsules

As the *L. acidophilus* TISTR 1338 were grown in both of prebiotics tested, individual prebiotic was further examined to co-encapsulate the probiotic bacteria by applying 3% prebiotic within 3% alginate matrix and coated microcapsules with 0.8% chitosan for protect cells under extreme condition. The results for viability and encapsulation efficiency of co-encapsulated with different prebiotic are shown in Table 2. Viable cells and encapsulation efficiency were nearly the same in each group. Previous studies showed that the co-encapsulation of different probiotic bacteria with Hi-maize starch (prebiotic) and further coating with chitosan significantly enhanced the survival of encapsulated probiotic bacteria. Furthermore, co-encapsulated probiotic bacteria with Hi-maize also survived better than the encapsulated bacteria without the prebiotic (Iyer and Kailasapathy, 2005). As the number of cells in control treatment which no prebiotic in microcapsules, shown the significantly decrease after freeze-drying process.
Table 2. Number of cells entrapped and encapsulation efficiency of encapsulated *L. acidophilus* TISTR 1338 after co-encapsulation with different prebiotics.

<table>
<thead>
<tr>
<th>Type of prebiotic</th>
<th>Number of cells (log cfu/ml)</th>
<th>EE (%)</th>
<th>%Survival after freeze-drying process</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial cells</td>
<td>Encapsulated cells</td>
<td>Freeze-dry encapsulated cells</td>
</tr>
<tr>
<td>Control (no prebiotic)</td>
<td>10.18±0.03</td>
<td>9.05±0.06</td>
<td>6.15±0.05</td>
</tr>
<tr>
<td>Inulin</td>
<td>9.83±0.20</td>
<td>8.84±0.26</td>
<td>7.32±0.41</td>
</tr>
<tr>
<td>Jerusalem artichoke</td>
<td>9.77±0.21</td>
<td>8.75±0.08</td>
<td>7.53±0.34</td>
</tr>
</tbody>
</table>

*ab* Means±standard deviation with different superscript in the same column are significantly different (P<0.05) according to DMRT.

Viability of microcapsules after freeze-drying

Low temperature and water sublimation that occur during freeze-drying are less harmful to microorganisms than the higher temperature used in spray-drying (Oliveira et al., 2007). Therefore, the viability of encapsulated cells with different prebiotic in alginate matrix coated with chitosan were analysed after freeze-dried process. The results showed that the survival rate of encapsulated *L. acidophilus* TISTR 1338 with inulin® and JA were significantly (P<0.05) higher than control treatment (without prebiotic microcapsule) as shown in Table 2. The co-encapsulated bacteria survived very well in prebiotic treatment as the previous studied, the addition of FOS provided the best protection during freeze-drying of *L. reuteri* TMW1 (Schwab et al., 2007). The application of sucrose and maltodextrins was previously shown to improve the viability of bacteria during drying and revealed that the direct interaction of sucrose with membranes was suggested to contribute to the protective effects of sucrose on dried cells of *Lactobacillus bulgaricus* (Oldenhof et al., 2005). During freeze-drying intracellular ice formation reduces the cell viability due to membrane injury. Therefore, interaction between the cells membrane and fructans of the FOS maintains the membrane fluidity (Schwab et al., 2007). Besides, Leslie et al. (1995) suggested that disaccharides such as trehalose and sucrose are able to prevent membrane damage and maintain the structure of proteins and biomolecules, limit the intracellular mobility of vital structures and maintain the function of these structures during the freezing stage. Ours studies revealed that co-encapsulation with Jerusalem artichoke which is rich in FOS and inulin may provide better protection and increase survivability for probiotic from stress during freeze-drying process.

Viability of microcapsules after heat treatment

In order to investigate the efficacy of microcapsules for protecting probiotic cells in food of feed processing which often employs high temperature. The survival rate of encapsulated *L. acidophilus* TISTR 1338 were evaluated after ex-
posed into feed machine and hot air oven at 70 °C for 30 min. The results showed that microcapsules without prebiotic were the most sensitive to heat compare to the survival rate of the co-encapsulated cells with JA and inulin® were 77.27% and 63.48%. In addition, the survival rate of co-encapsulated cells with JA showed significant higher value than inulin® (Table 3). Wada et al. (2005) reported that long-chain inulin® with a high degree of polymerization show high thermal stability and low solubility, which might have led to slower diffusion of the suspending medium, thus rendering higher protection of the bifidobacteria. Microencapsulation of Bifidobacterium bifidum with fructooligosaccharides as a prebiotic compound resulted in the highest heat-tolerance at 76°C for 1 min as the cells loss was less than 0.5 log (Chen et al., 2007). The variation in the composition of JA such as the degree of polymerization, sugar and glycosidic linkage, degree of branching and might be the reason for survivability of encapsulated with prebiotic which were damage suffered during heat processing. Incorporating both prebiotics and alginate-chitosan coating materials for encapsulate probiotic may better protect probiotic in food systems due to synbiosis (Chen et al., 2005; Nazzaroa et al., 2009).

**Table 3.** Survival rate of encapsulated L. Acidophilus TISTR 1338 after heat processing.

<table>
<thead>
<tr>
<th>Type of prebiotic</th>
<th>%Survival after heat processing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (no prebiotic)</td>
<td>43.34±3.84a</td>
</tr>
<tr>
<td>Inulin®</td>
<td>63.48±5.32b</td>
</tr>
<tr>
<td>Jerusalem artichoke</td>
<td>77.27±2.80c</td>
</tr>
</tbody>
</table>

a, b, c Means±standard deviation with different superscript in the same column are significantly different (P<0.05) according to DMRT.

**Conclusions**

Regarding to the concept of synbiotic which is a mixture of probiotics and prebiotics that synergistically enhance equilibrium of the gastrointestinal microflora, finding new natural resources containing various prebiotic components could be an appropriate way to develop food industry and improve host health. The results of this study indicated that co-encapsulation of probiotic L. acidophilus TISTR 1338 with Jerusalem artichoke and inulin® in alginate matrix coated with chitosan increased bacterial survival after freeze-drying process. Moreover, microcapsules exhibited higher survival rate after exposure to high temperature compared with none of prebiotic microcapsules. Jerusalem artichoke being used as prebiotics could improve viability of L. acidophilus from the stress condition. Therefore, co-encapsulation technique might be useful to develop and apply into food and feed industry instead of antibiotics to improve the host’s health.

**Acknowledgements**

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