RESEARCH ARTICLE
doi: 10.2306/scienceasia1513-1874.2023.080

ScienceAsia 49 (2023): **765–775**

High survivability of microencapsulated canine-specific probiotics during artificial gastrointestinal conditions and pasteurization

Ngamlak Foongsawat[a], Sirinthorn Sunthornthummas[b], Achariya Rangsiruji[c], Siriruk Sarawaneeyaruk[a], Kedwadee Insian[a], Onanong Pringsulaka[a,*]

[a] Department of Microbiology, Faculty of Science, Srinakharinwirot University, Bangkok 10110 Thailand
[b] National Biobank of Thailand (NBT), National Science and Technology Development Agency, Pathum Thani 12120 Thailand
[c] Department of Biology, Faculty of Science, Srinakharinwirot University, Bangkok 10110 Thailand

[*]Corresponding author, e-mail: onanong@g.swu.ac.th

Received 3 May 2023, Accepted 2 Aug 2023
Available online 12 Oct 2023

ABSTRACT: Probiotics are beneficial bacteria that can positively impact the health and well-being of host organisms. When selecting probiotics for canines, it is crucial to choose species that are native to the canine intestinal tract, as these species demonstrate host specificity. In this study, two promising lactic acid bacteria (LAB) strains, *Limosilactobacillus fermentum* Pom5 and *Pediococcus pentosaceus* Chi8, were microencapsulated using the extrusion method with sodium alginate (SA) and sodium alginate-goat milk (SAGM) matrices. The results showed that the highest encapsulation yield was observed in SAGM microbeads. The surface of SAGM microbeads exhibited a smooth wavy appearance with a denser and layered structure. The viability of both microencapsulated and non-encapsulated LAB cells was assessed under various conditions, including simulated gastrointestinal (GI) conditions, refrigeration, storage in goat milk at 4 °C for 28 days, and incubation under pasteurization temperature. The SAGM microbeads demonstrated the highest survival rate with viable cell counts consistently exceeding 6 log cfu/g when subjected to individual and sequential artificial GI conditions as well as other tested conditions. This sequential assessment provided a more realistic representation of the complex gastrointestinal environment. The maximum release rates of the SA and SAGM microbeads were 86.61% and 85.01%, respectively, after 6 h of incubation. This indicates that the encapsulated bacteria were gradually released from the microbeads over time. The study suggests that both SA and SAGM are suitable matrices for encapsulating the two strains of bacteria, but SAGM showed particular promise as an effective encapsulation material for probiotic bacteria.

KEYWORDS: lactic acid bacteria, probiotic, dogs, microencapsulation, goat milk

INTRODUCTION

The intestinal microbiota plays a pivotal role in maintaining the optimal health and well-being of dogs [1, 2]. However, an imbalance or reduction in diversity of the microbiota has been associated with various diseases, including inflammation, metabolic syndromes, obesity, and mood dysfunction [3]. Therefore, regulating the colonic microbiota has become increasingly important in improving the dog's health. Among the various methods used to regulate the intestinal microbiota and promote gut health, the supplementation of probiotics, prebiotics, or a combination of both has shown promising results in improving the health status of dogs [4].

Probiotics are living microorganisms that, when consumed in adequate quantities, provide health benefits to the host [5]. Probiotics promote health by directly inhibiting the growth of harmful bacteria in the gut through various mechanisms such as the production of antimicrobial substances, competition for nutrients and space, and stimulation of the immune system. Probiotics may also help to improve the overall immune function of the host by enhancing the gut-associated lymphoid tissue, which plays a crucial role in regulating the immune response [6]. Lactic acid bacteria (LAB) are often used as probiotics due to their natural presence in the gut and their generally recognized safety for consumption. However, it is important to note that not all strains of LAB are suitable for use as probiotics, and more research is needed to identify and select the most effective strains for specific health benefits [7–9]. However, the viability of LAB decreases during the manufacturing process and exposure to gastric and bile acids in the digestive tract, which reduces the benefits of probiotics. Therefore, there is a need to enhance the viability of LAB both during the manufacturing process and within the gastrointestinal (GI) tract [10, 11].

Microencapsulation is often applied to probiotic products to maintain the viability of the bacteria and protect them during processing and passage through the digestive tract. Various microencapsulation techniques, especially spray drying, freeze drying, emulsion, and extrusion, have been used with probiotics to improve their survival rates during the adverse

conditions of GI transit [12]. However, certain microencapsulating methods can potentially affect the viability and performance of microencapsulated probiotic bacteria. Among these techniques, extrusion has been reported to involve milder conditions and is well-known for its effectiveness in the encapsulation of probiotics [13]. Sodium alginate (SA) is a commonly used material for encapsulating probiotics. However, a disadvantage of alginate beads is that their porous structure may not provide sufficient protection for probiotic bacteria in highly acidic environments [14]. Hence, it is advisable to incorporate alginate with additional protective materials to enhance the effectiveness of the encapsulation and increase the viability rates of probiotic bacteria [15].

Numerous research studies have shown the efficacy of different matrices based on alginate for microencapsulation of probiotics, including chitosan, zein, gum arabic, cellulose, starch, whey protein, gelatin, and pectin [16] as well as alginate-dairy-based matrices [17]. Alginate-dairy-based matrices have shown particular promise as encapsulation materials for probiotic bacteria. These matrices consist of carbohydrates, fats, and proteins, which can contribute to the enhanced viability and survival of the microencapsulated probiotics [18]. When considering different milk options for dogs and pets, it is advisable to choose goat milk due to its lower lactose content and improved digestibility compared to cow milk. Goat milk typically contains approximately 4.2–4.8% lactose, whereas cow's milk contains around 4.7–5.0% lactose [19]. Moreover, goat milk provides additional benefits such as prebiotics and probiotics, which can effectively enhance the health of dogs. However, it is worth noting that the efficacy of alginate-goat milk-based matrices in encapsulating probiotics specifically for dogs has not been reported.

For this attempt, the most promising lactic acid bacteria strains from canine feces were previously screened for probiotic attributes and evaluated for safety properties through a combination of genome analyses and phenotypic tests [20]. Among the strains, we identified 2 LAB strains, namely *Limosilactobacillus fermentum* Pom5 and *Pediococcus pentosaceus* Chi8, which displayed the best probiotic properties. The aim of this study was to microencapsulate these 2 probiotic LAB strains by employing the extrusion technique in accordance with 2 different matrices: SA and sodium alginate-goat milk (SAGM). The encapsulation yield, bead size, and surface structure of the microcapsules were also assessed. Additionally, the viability of both microencapsulated and non-encapsulated LAB cells was investigated under artificial GI conditions, during storage in refrigeration and goat milk at 4 °C for 28 days as well as when incubated under pasteurization temperature.

MATERIALS AND METHODS

LAB strains and culture condition

Lim. fermentum Pom5 and *P. pentosaceus* Chi8 were obtained from our previous study [20]. Both strains were routinely cultivated on de Man, Rogosa, and Sharpe (MRS) agar (Himedia, India) under microaerophilic conditions at 37 °C.

Encapsulation of LAB strains

The encapsulation of LAB strains in SA and SAGM matrices was carried out using the method described by Prasanna and Charalampopoulos [17]. Briefly, a solution of 2% w/v sodium alginate (Himedia) and SAGM (sodium alginate to goat milk ratio of 2:1, v/v) was prepared. The concentrated cell suspension was mixed with the alginate-based mixture solution (alginate or alginate-goat milk solution to concentrated cell suspension ratio of 4:1, v/v). These mixtures were then dropped into sterile 0.5 M $CaCl_2$ (Thermo Fisher Scientific, USA) using a 21G needle (Nippo, Thailand) with gentle stirring. After the microbeads were set, they were washed with sterile 0.85% NaCl solution and then stored in sterilized bottles at 4 °C. The cell number in the microbeads was approximately 9 log cfu/g. For non-encapsulated cells, 10 ml of the concentrated cell suspension was combined with 40 ml of sterile 0.85% NaCl solution.

Determination of size and encapsulation yield of alginate milk-based microbeads

The size of the alginate-milk microbeads was determined using a vernier caliper. For this purpose, 30–40 microbeads were randomly selected and measured to calculate the mean size [18]. The size of the microbeads was expressed as the mean diameter in micrometers (μm) ± standard deviation (SD).

The encapsulation yield was calculated by determining the percentage of cells microencapsulated in the microbeads relative to the total initial cell count. To obtain the microencapsulated cell count, a known weight of microbeads was dissolved in sterile 0.85% NaCl, and the released cells were enumerated by viable plate count on MRS agar medium. The encapsulation yield (EY) was calculated according to the following formula: Encapsulation yield (%) = (Microencapsulated cell count/Total initial cell count)×100.

Determination of survivability of non-encapsulated and microencapsulated bacteria

The viability of both free and microencapsulated bacteria was determined using the following methods. For free cells, serial dilutions were prepared using sterile saline, and then 0.1 ml aliquots of the diluted samples were spread onto MRS agar to determine viable cells. The plates were then incubated at 37 °C for 72 h. For microencapsulated bacteria, the samples were completely solubilized in sterilized 50 mM

sodium citrate solution (Himedia) at pH 7.5, following the method described by Shi et al [13]. Briefly, 1 g of the microencapsulated matrix was mixed with 9 ml of sodium citrate solution and then serially diluted in sterile 0.85% NaCl. This process allowed for the assessment of the survival and release of bacteria from microbeads. The viable count was determined by plating 0.1 ml of the serially diluted sample on MRS agar, followed by incubation at 37 °C and enumeration of viable cells.

Survivability of non-encapsulated and microencapsulated bacteria *in vitro* artificial GI conditions

The preparation and evaluation of artificial saliva fluid (ASF), artificial gastric juice (AGJ), and artificial intestinal juice (AIJ) as well as the enumeration of viable cells were carried out as follows:

ASF, containing 0.77 g of 100 U α-amylase in 0.85% NaCl (w/v), was prepared as described by Bao et al [21]. The α-amylase solution was filtered through a 0.45-µm filter. Microbeads (1 g) or free cells (1 ml) were mixed with 9 ml of sterilized ASF and incubated at 37 °C for 5 min. Samples were collected during incubation. The free and microencapsulated LAB were enumerated following the method as described previously.

AGJ, containing 3 g of pepsin in 0.2% NaCl (w/v), pH 2.0, was prepared [22]. Microbeads (1 g) or free cells (1 ml) were mixed with 9 ml of sterilized AGJ and incubated 37 °C for 120 min. For free cells, the samples were collected and centrifuged at 10,000×*g* for 15 min at 4 °C. The resulting pellet was then diluted in sterile 0.85% NaCl and enumerated. The free and microencapsulated LAB were enumerated following the method as described previously.

AIJ was prepared by dissolving 3 g of bile salt (Himedia) in 1,000 ml of intestinal solution containing 6.5 g/l NaCl, 0.835 g/l KCl, 0.22 g/l $CaCl_2$, and 1.386 g/l $NaHCO_3$ [23]. Microbeads (1 g) or free cells (1 ml) were mixed with 9 ml of sterilized AIJ and incubated at 37 °C for 180 min. Sampling and enumeration of free and microencapsulated LAB were carried out using the previously described method.

Survivability of non-encapsulated and microencapsulated bacteria during sequential incubation *in vitro* artificial digestive tract conditions

The survival of both free and microencapsulated bacterial cells during sequential incubation in artificial GI conditions was evaluated as described previously [24] with the inclusion of an additional step involving ASF. Briefly, 1 g of microbeads or 1 ml of free cells were added to 9 ml of ASF and incubated for 5 min. After incubation, the treated microbeads and free cells were centrifuged at 10,000×*g* to remove the ASF, and the cells or microbeads were then transferred into 10 ml of AGJ. The microbeads and free cell samples were further incubated for 180 min. Subsequently, the treated microbeads and free cells were centrifuged at 10,000×*g* to remove the AGJ, and the cells or microbeads were transferred into 10 ml of AIJ, followed by incubation for 360 min. All incubations were performed with shaking at 100 rpm and at a temperature of 37 °C. The viability of the free cells and microencapsulated LAB before and after exposure to artificial GI conditions was determined by plating on MRS agar using methods previously described.

Release profiles of microencapsulated probiotic cells in artificial intestinal condition

The rate of release of microencapsulated probiotics from SA and SAGM matrices was studied by adding microbeads to AIJ and incubating at 37 °C for 6 h. One ml aliquots were taken at each time point, serially diluted in sterile 0.85% NaCl, and spread onto MRS agar. After incubation for 24 h at 37 °C, colonies were counted to determine the number of released cells.

Survivability of non-encapsulated and microencapsulated bacteria during refrigeration and in the presence of goat milk at 4 °C

The viability of non-encapsulated and microencapsulated bacteria was investigated under refrigeration conditions and when submerged in goat milk at 4 °C. In this study, 1 g of microbeads or 1 ml of free cells was placed in sterile centrifuge tubes (NEST, China) and stored at 4 °C for 28 days. For goat milk, 10 ml of sterilized goat milk were added to 1 g of microencapsulated cells or 1 ml of free cells in centrifuge tubes and then stored at 4 °C for 28 days. Samples were taken on days 0, 7, 14, 21, and 28, and cell viability was determined using the method described above.

Structure of microbeads using scanning electron microscopy (SEM)

The microbeads were dehydrated using a sequential series of ethanol solutions and then fixed to SEM stubs using adhesive tape. Subsequently, the microbeads were coated with gold using a sputter coater SCD 040 (Balzers, Germany) following the method described by Prasanna and Charalampopoulos [17]. Finally, the coated microbeads were examined using a scanning electron microscope JSM-IT300 (JEOL InTouchScope, USA).

Microencapsulated cell survivability under pasteurization temperature

This study followed the method described by Mao et al [25]. Briefly, 1 g of microbeads or 1 ml of free cells was added into sterile goat milk and incubated at 63 °C for 30 min and then at 72 °C for 15 s. The sample was quickly cooled and analyzed for cell viability as described previously.

Statistical analysis

All experiments were conducted in triplicate. The size of microbeads and encapsulation yield results were analyzed using Student's *t*-test analysis. Viable cell counts from artificial GI conditions, storage, and pasteurization temperature studies were analyzed using one-way analysis of variance (ANOVA) with Tukey's multiple comparison tests in SAS (version 9.2, SAS Institute Inc., Cary, NC, USA).

RESULTS

Determination of size, encapsulation yield, and structure of alginate and alginate-goat milk microbeads

The size of the different microbeads was shown in Table 1. The mean size of SA and SAGM microbeads was about 2.2 mm. When comparing the size of SA and SAGM microbeads, significant differences were observed ($p \leqslant 0.05$) as shown in Table 1. Additionally, the encapsulation yield of *P. pentosaceus* Chi8 in SA and SAGM microbeads also showed significant differences. These findings suggest that SAGM microbeads had higher encapsulation yield compared to SA microbeads. The highest encapsulation yield was observed in SAGM microbeads microencapsulated with *P. pentosaceus* Chi8.

The surface morphology of the microbeads was examined using SEM micrographs, as shown in Fig. 1. At a magnification of 10,000 ×, the surface of SAGM microbeads exhibited a smooth wavy appearance with a denser and layered structure (Fig. 1C and 1D), whereas SA microbeads appeared as single layer patches (Fig. 1A and 1B).

Survivability of free and microencapsulated bacteria *in vitro* artificial GI conditions

Survivability of non-encapsulated and microencapsulated bacteria in artificial saliva fluid

Microencapsulation strategies were evaluated in artificial oral phases with free cells used as a control. In the oral phase, survival rates showed no significant decrease in all treatment groups (Table 2). Therefore, our results indicate that SA, SAGM, and free cells were stable enough under higher salivary α-amylase concentrations with survival rates of 97.83–99.55% when incubated for 5 min. Although specific data on the duration of the oral phase in dogs are not available, it is widely recognized that dogs tend to quickly swallow their food without prolonged oral processing [26]. Consequently, it can be inferred that the duration of our study aligns with the average oral duration of food in dogs.

Survivability of non-encapsulated and microencapsulated bacteria in artificial gastric juice

Microencapsulation significantly protected cells in AGJ as shown in Table 3. The viable cell counts of microencapsulated cells of the 2 LAB strains were still detected in the range of 6.95–7.57 log cfu/ml after 180 min, whereas the cell counts of free cells were about 5.62–5.87 log cfu/ml after 180 min. When comparing the different matrices SA and SAGM, the SAGM capsule provided higher protection for viable cells than SA with a statistically significant difference ($p \leqslant 0.05$). The highest viable cell counts of both microencapsulated LAB strains, as shown in SAGM, were 7.57 and 7.37 log cfu/g after 180 min, respectively. The present study also demonstrated that both SA and SAGM matrices were effective in protecting both LAB strains from the highly acidic environment for an extended period of time.

Survivability of non-encapsulated and microencapsulated bacteria in artificial intestinal juice

The survival of free and microencapsulated LAB in AIJ at 37 °C for 180 min is presented in Table 3. Encapsulation with SA and SAGM matrices provided better protection for bacterial cells in AIJ compared to free cells with SAGM showing higher protection than SA. In addition, the viable count of non-encapsulated bacteria showed a decrease of about 1.46–1.78 log cfu/ml within 180 min.

Survivability of non-encapsulated and microencapsulated bacteria during sequential incubation in vitro artificial GI conditions

The survival of both free cells and microencapsulated LAB strains during sequential incubation in artificial GI conditions is presented in Table 4. Similar trends were observed in artificial saliva, gastric, and intestinal juices. Encapsulation in both alginate and alginate-goat milk significantly improved the survival of LAB cells compared to free cells. Notably, microencapsulated LAB cells using SAGM exhibited the highest survival with capsule stability maintained even after 360 min of exposure to artificial GI conditions.

Release profiles of microencapsulated probiotic cells in an artificial intestinal juice condition

The release profiles of microencapsulated *Lim. fermentum* Pom5 and *P. pentosaceus* Chi8 using SA and SAGM under AIJ conditions were evaluated, and the results are presented in Table 5. It was observed that the microencapsulated *Lim. fermentum* Pom5 and *P. pentosaceus* Chi8 using SA and SAGM exhibited slow release upon transfer into the AIJ. After 6 h of incubation, the maximum release rates of SA for *Lim. fermentum* Pom5 and *P. pentosaceus* Chi8 were 86.61%

Table 1 Encapsulation yield and size of microbeads in different LAB strains.

LAB strain	Size (mm)		Encapsulation yield (%)	
	SA	SAGM	SA	SAGM
Lim. fermentum Pom5	2.24 ± 0.02^{B}	2.26 ± 0.01^{A}	94.05 ± 0.29^{A}	96.64 ± 0.81^{A}
P. pentosaceus Chi8	2.21 ± 0.02^{B}	2.29 ± 0.01^{A}	92.27 ± 0.16^{B}	98.97 ± 0.73^{A}

The values presented represent the mean ± standard deviation of 3 determinations. Different capital letters indicate statistically significant differences between different matrices (rows) within each strain with a significance level of $p \leqslant 0.05$ (Student's *t*-test).

Table 2 Survivability of non-encapsulated and microencapsulated LAB in artificial saliva fluid at 37 °C for 5 min.

LAB strain	Encapsulation material	Incubation period (min)[†]		
		0	5	Survival rate (%)[‡]
Lim. fermentum Pom5	SA	7.76 ± 0.05^{A}	7.65 ± 0.06^{A}	99.36^{A}
	SAGM	7.83 ± 0.05^{A}	7.79 ± 0.17^{A}	97.83^{A}
	non-encapsulated	7.66 ± 0.07^{A}	7.67 ± 0.08^{A}	98.20^{A}
P. pentosaceus Chi8	SA	7.79 ± 0.03^{A}	7.77 ± 0.07^{A}	98.45^{A}
	SAGM	7.73 ± 0.06^{A}	7.70 ± 0.09^{A}	98.52^{A}
	non-encapsulated	7.63 ± 0.03^{A}	7.62 ± 0.03^{A}	99.55^{A}

Values represent mean ± standard deviation of 3 determinations. Statistically significant differences within matrices are indicated by different capital letters (columns) at each time point with a significance level of $p \leqslant 0.05$ (Tukey's test).
[†] Log cfu/g for microencapsulated cells or log cfu/ml for non-encapsulated cells.
[‡] Calculated by dividing the final viable cells (cfu/g or cfu/ml) by initial viable cells (cfu/g or cfu/ml) of LAB.

Table 3 Survivability of non-encapsulated and microencapsulated LAB in artificial gastric juice (pH 2) at 37 °C for 180 min and in artificial intestinal juice (pH 7.4) at 37 °C for 180 min.

In artificial gastric juice (pH 2) at 37 °C for 180 min									
LAB strain	Encapsulation material	Incubation period (min)[†]							Survival rate (%)[‡]
		0	30	60	90	120	150	180	
Lim. fermentum Pom5	SA	8.07 ± 0.05^{C}	7.93 ± 0.06^{B}	7.77 ± 0.05^{B}	7.34 ± 0.04^{B}	7.96 ± 0.07^{A}	7.22 ± 0.08^{B}	7.01 ± 0.10^{B}	86.86^{A}
	SAGM	8.63 ± 0.07^{B}	8.56 ± 0.19^{A}	7.98 ± 0.05^{A}	7.86 ± 0.03^{A}	7.99 ± 0.11^{A}	7.78 ± 0.08^{A}	7.57 ± 0.21^{A}	87.72^{A}
	Non-encapsulated	8.87 ± 0.06^{A}	7.81 ± 0.06^{B}	7.62 ± 0.07^{B}	7.02 ± 0.10^{C}	6.48 ± 0.05^{B}	5.96 ± 0.09^{C}	5.87 ± 0.07^{C}	66.12^{B}
P. pentosaceus Chi8	SA	8.17 ± 0.02^{B}	7.81 ± 0.02^{B}	8.05 ± 0.08^{B}	7.77 ± 0.10^{B}	7.52 ± 0.21^{B}	7.54 ± 0.07^{B}	6.95 ± 0.08^{B}	85.05^{A}
	SAGM	8.35 ± 0.08^{A}	8.17 ± 0.06^{A}	8.10 ± 0.06^{A}	7.97 ± 0.04^{A}	8.28 ± 0.06^{A}	7.75 ± 0.07^{A}	7.37 ± 0.23^{A}	88.23^{A}
	Non-encapsulated	8.89 ± 0.03^{A}	7.48 ± 0.02^{C}	7.24 ± 0.07^{C}	6.87 ± 0.06^{C}	6.21 ± 0.07^{C}	5.95 ± 0.06^{C}	5.62 ± 0.08^{C}	63.20^{B}
In artificial intestinal juice (pH 7.4) at 37 °C for 180 min									
LAB strain	Encapsulation material	Incubation period (min)[†]							Survival rate (%)[‡]
		0	30	60	90	120	150	180	
Lim. fermentum Pom5	SA	8.40 ± 0.04^{B}	8.34 ± 0.02^{B}	8.18 ± 0.03^{B}	7.91 ± 0.10^{B}	7.87 ± 0.02^{C}	7.78 ± 0.03^{B}	7.31 ± 0.15^{B}	87.06^{A}
	SAGM	8.51 ± 0.08^{B}	8.43 ± 0.06^{AB}	8.39 ± 0.02^{A}	8.24 ± 0.08^{A}	8.21 ± 0.02^{A}	8.03 ± 0.05^{A}	7.63 ± 0.22^{A}	89.71^{A}
	Non-encapsulated	8.83 ± 0.03^{A}	8.53 ± 0.03^{A}	8.35 ± 0.04^{A}	8.13 ± 0.08^{A}	8.06 ± 0.05^{B}	7.66 ± 0.09^{B}	7.05 ± 0.01^{C}	79.86^{B}
P. pentosaceus Chi8	SA	8.12 ± 0.05^{C}	8.07 ± 0.04^{C}	7.98 ± 0.03^{B}	7.84 ± 0.09^{B}	7.90 ± 0.05^{B}	7.71 ± 0.05^{B}	7.72 ± 0.02^{B}	94.99^{A}
	SAGM	8.83 ± 0.03^{A}	8.72 ± 0.02^{A}	8.49 ± 0.03^{A}	8.33 ± 0.10^{A}	8.39 ± 0.03^{A}	8.05 ± 0.07^{A}	8.09 ± 0.05^{A}	91.62^{A}
	Non-encapsulated	8.61 ± 0.02^{B}	8.26 ± 0.03^{B}	8.11 ± 0.07^{B}	7.97 ± 0.06^{B}	7.82 ± 0.04^{B}	7.49 ± 0.10^{C}	7.15 ± 0.04^{C}	83.04^{C}

Values represent mean ± standard deviation of 3 determinations. Statistically significant differences within matrices are indicated by different capital letters (columns) at each time point with a significance level of $p \leqslant 0.05$ (Tukey's test).
[†] Log cfu/g for microencapsulated cells or log cfu/ml for non-encapsulated cells.
[‡] Calculated by dividing the final viable cells (cfu/g or cfu/ml) by initial viable cells (cfu/g or cfu/ml) of LAB.

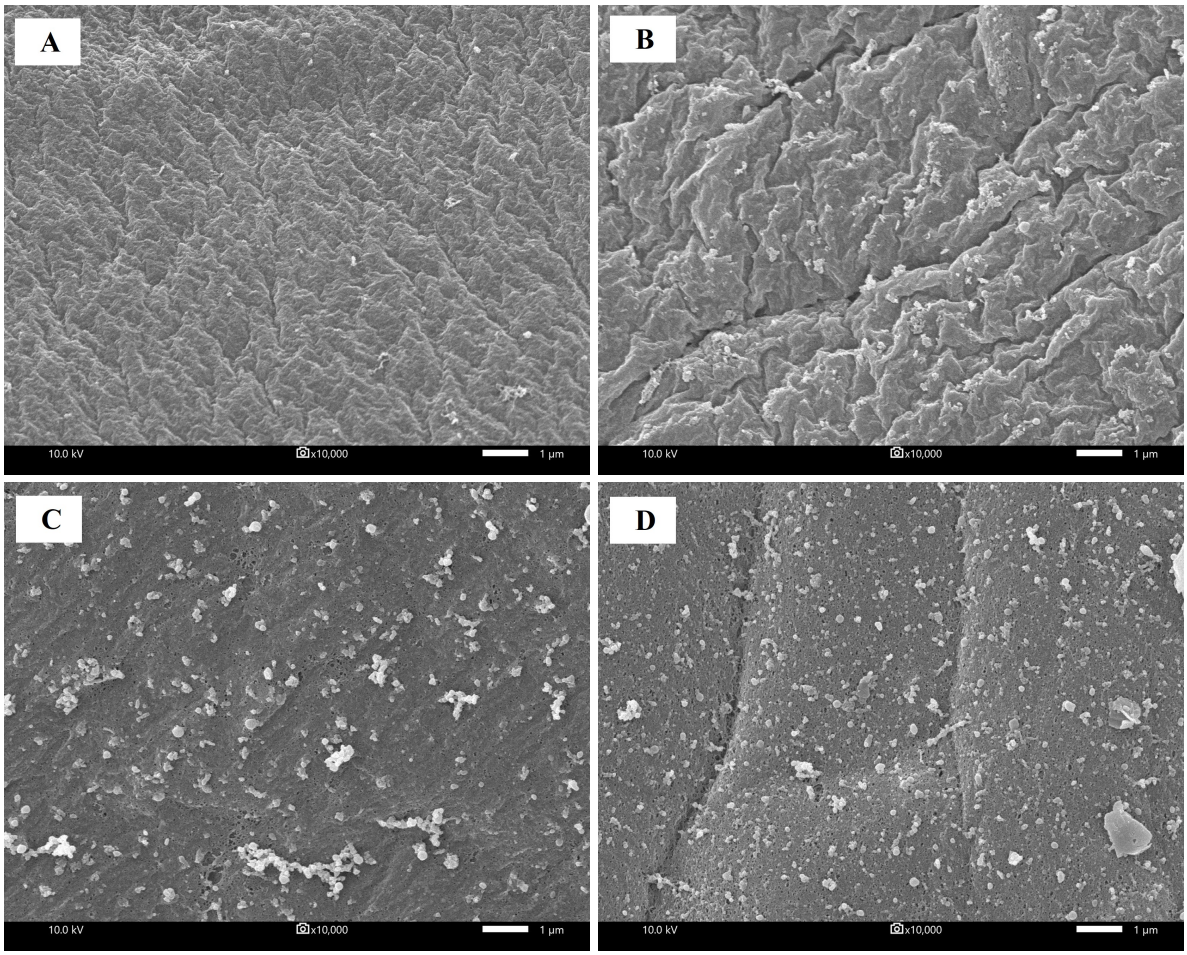

Fig. 1 Surface morphology of microbeads using Scanning electron microscopy: (A) SA microbeads, (B) SA microbeads microencapsulated with *Lim. fermentum* Pom5, (C) SAGM microbeads, and (D) SAGM microbeads microencapsulated with *Lim. fermentum* Pom5 (magnification 10,000×).

Table 4 Survivability of non-encapsulated and microencapsulated probiotic bacteria during sequential incubation in artificial digestive tract conditions at 37 °C for 360 min.

LAB strain	Encapsulation material	Incubation time (min)[†]				
		Artificial saliva fluid		Artificial gastric juice	Artificial intestinal juice	Survival rate (%)[‡]
		0	10	180	360	
Lim. fermentum Pom5	SA	9.35 ± 0.17^{A}	9.19 ± 0.17^{A}	6.54 ± 0.07^{B}	5.70 ± 0.06^{B}	61.02^{B}
	SAGM	9.34 ± 0.19^{A}	9.29 ± 0.13^{A}	7.38 ± 0.08^{A}	6.86 ± 0.06^{A}	73.70^{A}
	Non-encapsulated	9.50 ± 0.06^{A}	9.28 ± 0.13^{A}	5.64 ± 0.11^{C}	4.36 ± 0.06^{C}	45.88^{C}
P. pentosaceus Chi8	SA	9.15 ± 0.05^{B}	9.07 ± 0.04^{B}	6.60 ± 0.22^{B}	5.88 ± 0.06^{B}	64.25^{A}
	SAGM	9.22 ± 0.08^{B}	9.16 ± 0.08^{B}	7.29 ± 0.14^{A}	6.48 ± 0.29^{A}	70.22^{A}
	Non-encapsulated	9.35 ± 0.17^{A}	9.19 ± 0.17^{A}	6.54 ± 0.07^{B}	5.70 ± 0.06^{B}	61.02^{B}

Values represent mean ± standard deviation of 3 determinations. Statistically significant differences within matrices are indicated by different capital letters (columns) at each time point with a significance level of $p \leqslant 0.05$ (Tukey's test).

[†] Log cfu/g for microencapsulated cells or log cfu/ml for non-encapsulated cells.

[‡] Calculated by dividing the final viable cells (cfu/g or cfu/ml) by initial viable cells (cfu/g or cfu/ml).

Table 5 Release rate of microencapsulated probiotic cells in an artificial intestinal juice condition.

LAB strain	Encapsulation material	Incubation time (h)† 0	1	2	3	4	5	6
Lim. fermentum Pom5	SA	64.84 ± 0.10^{A}	67.29 ± 0.10^{B}	69.33 ± 0.05^{B}	75.18 ± 0.05^{A}	78.68 ± 0.02^{A}	82.44 ± 0.05^{A}	86.61 ± 0.01^{A}
	SAGM	65.80 ± 0.03^{A}	69.19 ± 0.07^{A}	72.40 ± 0.06^{A}	74.79 ± 0.06^{A}	77.45 ± 0.06^{A}	80.42 ± 0.09^{A}	85.01 ± 0.03^{A}
P. pentosaceus Chi8	SA	60.96 ± 0.06^{A}	66.99 ± 0.09^{A}	73.72 ± 0.47^{A}	73.53 ± 0.52^{A}	77.87 ± 0.53^{A}	78.82 ± 0.33^{A}	81.93 ± 0.20^{A}
	SAGM	61.18 ± 0.14^{A}	71.59 ± 0.13^{A}	70.70 ± 0.50^{B}	72.63 ± 0.31^{A}	75.47 ± 0.18^{A}	77.15 ± 0.41^{A}	81.23 ± 0.18^{A}

Values represent mean ± standard deviation of 3 determinations. Statistically significant differences within matrices are indicated by different capital letters (columns) at each time point with a significance level of $p \leqslant 0.05$ (Student's t-test).
‡ Log cfu/g for microencapsulated cells or log cfu/ml for non-encapsulated cells.

and 81.93%, respectively, while the maximum release rates of SAGM for *Lim. fermentum* Pom5 and *P. pentosaceus* Chi8 were 85.01% and 81.23%, respectively. No significant difference was observed in the release profiles of both LAB strains when microencapsulated with SA and SAGM.

Survivability of non-encapsulated and microencapsulated bacteria during refrigeration

Table 6 shows the viability of free and microencapsulated LAB with different capsules during the refrigerated storage at 4 °C. During 28 days of storage, the viable counts of free cells decreased by approximately 2.35 and 2.03 log cfu/ml for *Lim. fermentum* Pom5 and *P. pentosaceus* Chi8, respectively. The viability of cells in SA capsules decreased by about 2.02 and 1.95 log cfu/ml for *Lim. fermentum* Pom5 and *P. pentosaceus* Chi8, respectively, which was not significantly different from the free cells. However, the cell viability in SAGM capsules was significantly higher compared to that in both the free cells and SA capsules during refrigerated storage. Moreover, all treatments were able to maintain cell viability above the recommended count of 6 log cfu/g after 28 days of storage.

Survivability of non-encapsulated and microencapsulated bacteria in goat milk during refrigerated storage

The survival results of free and microencapsulated cells stored in goat milk at 4 °C for 28 days were presented in Table 6. The findings indicate that encapsulation improved the survival of bacterial cells in goat milk during storage. The free cells showed a survival rate in goat milk ranging from 80.36% to 84.44% with a reduction in cell concentration of 1.44 to 1.84 log cfu/ml after 28 days of storage. SAGM microbeads provided greater cell protection compared to SA and free cells, although the difference was not statistically significant. Moreover, all treatments demonstrated the ability to maintain the viability level exceeding 6 log cfu/g in goat milk throughout the duration of storage. Additionally, the survival rate of both free and microencapsulated cells in goat milk was improved compared to the absence of goat milk (Table 7). This result suggests that both free and microencapsulated cells with SA and SAGM in goat milk could be applied in feed products, not only providing sufficient amounts for host supply but also maintaining survivability during longer storage at 4 °C for 28 days.

Microencapsulated cell survivability under pasteurization temperature

Table 7 presents the viability of microencapsulated *Lim. fermentum* Pom5 and *P. pentosaceus* Chi8 cells at different pasteurization temperatures, compared to non-encapsulated cells. The viability of non-encapsulated *Lim. fermentum* Pom5 and *P. pentosaceus* Chi8 cells was found to significantly decrease after pasteurization at 63 °C for 15 min and 72 °C for 15 s. In contrast, encapsulation using SAGM maintained the survivability of *Lim. fermentum* Pom5 and *P. pentosaceus* Chi8 of approximately 7.22–7.94 log cfu/g and 6.33–7.37 log cfu/g, respectively. These results suggest that SAGM demonstrated efficacy in enhancing thermal stability of *Lim. fermentum* Pom5 and *P. pentosaceus* Chi8.

DISCUSSION

This study highlights the potential of microencapsulation as a promising approach to enhance the survivability of probiotics in conditions relevant to the GI tract and food processing. While probiotics are commonly used in human health, their use in dogs is comparatively rare due to various reasons such as limited research, regulatory constraints, and differences in digestive systems. Previous studies have successfully identified and isolated LAB from the feces of dogs, suggesting their potential as probiotics for dogs [27–29]. However, the encapsulation of probiotics derived from dogs has been scarce. Encapsulation of probiotics for dogs can offer several benefits, including enhanced viability, improved stability, controlled release, easy administration, customization, and increased safety. For this purpose, it is crucial to choose high-quality encapsulated probiotic products specifically formulated for dogs.

This study attempted to microencapsulate 2 selected probiotics that were isolated from dogs. Microbeads were prepared, and important parameters such as size, surface morphology, and encapsulation

Table 6 Changes in the viable count of non-encapsulated and the microencapsulated probiotic bacteria during refrigerated storage (4 °C) for 28 days and in goat milk at 4 °C for 28 days.

In refrigerated storage (4 °C) for 28 days							
LAB strain	Encapsulation material	Day of storage† 0	7	14	21	28	Survival rate (%)‡
Lim. Fermentum Pom5	SA	8.73 ± 0.20^{A}	8.22 ± 0.06^{B}	7.87 ± 0.08^{B}	7.09 ± 0.11^{B}	7.05 ± 0.10^{B}	80.74^{B}
	SAGM	8.90 ± 0.27^{A}	8.69 ± 0.40^{A}	8.54 ± 0.08^{A}	8.17 ± 0.06^{A}	8.10 ± 0.06^{A}	91.01^{B}
	Non-encapsulated	8.92 ± 0.04^{A}	8.86 ± 0.08^{A}	7.56 ± 0.40^{B}	6.50 ± 0.17^{B}	6.57 ± 0.21^{B}	73.58^{A}
P. pentosaceus Chi8	SA	8.81 ± 0.09^{A}	8.56 ± 0.05^{A}	7.71 ± 0.01^{B}	7.61 ± 0.06^{B}	7.06 ± 0.04^{B}	80.17^{B}
	SAGM	8.96 ± 0.09^{A}	8.78 ± 0.05^{A}	8.57 ± 0.02^{A}	8.14 ± 0.08^{A}	7.98 ± 0.08^{A}	89.06^{A}
	Non-encapsulated	8.89 ± 0.16^{A}	7.61 ± 0.24^{B}	6.82 ± 0.05^{C}	6.94 ± 0.09^{C}	6.55 ± 0.10^{C}	73.67^{C}
In goat milk at 4 °C for 28 days							
LAB strain	Encapsulation material	Day of storage† 0	7	14	21	28	Survival rate (%)‡
Lim. Fermentum Pom5	SA	8.87 ± 0.08^{A}	8.83 ± 0.25^{A}	8.31 ± 0.36^{A}	8.25 ± 0.29^{A}	7.80 ± 0.28^{A}	87.98^{A}
	SAGM	8.81 ± 0.34^{A}	8.65 ± 0.51^{A}	8.17 ± 0.29^{A}	8.18 ± 0.10^{A}	8.16 ± 0.54^{A}	92.66^{A}
	Non-encapsulated	9.03 ± 0.07^{A}	8.77 ± 0.24^{A}	8.41 ± 0.24^{A}	8.10 ± 0.14^{A}	7.92 ± 0.25^{A}	87.67^{A}
P. pentosaceus Chi8	SA	9.31 ± 0.22^{A}	9.01 ± 0.43^{A}	9.09 ± 0.39^{A}	8.96 ± 0.22^{A}	8.70 ± 0.57^{A}	93.44^{AB}
	SAGM	9.19 ± 0.40^{A}	9.23 ± 0.54^{A}	9.16 ± 0.37^{A}	8.97 ± 0.58^{A}	9.06 ± 0.53^{A}	98.66^{A}
	Non-encapsulated	9.68 ± 0.38^{A}	9.47 ± 0.11^{A}	8.86 ± 0.38^{A}	8.50 ± 0.11^{A}	8.11 ± 0.11^{A}	83.76^{B}

Values represent mean ± standard deviation of 3 determinations. Statistically significant differences within matrices are indicated by different capital letters (columns) at each time point with a significance level of $p \leqslant 0.05$ (Tukey's test).

† Log cfu/g for microencapsulated cells or log cfu/ml for non-encapsulated cells.

‡ Calculated by dividing the final viable cells (cfu/g or cfu/ml) by initial viable cells (cfu/g or cfu/ml).

Table 7 Changes in the viable count of non-encapsulated and the microencapsulated probiotic bacteria in pasteurization temperature.

LAB strain	Encapsulation material	Before pasteurization	After pasteurization temperature (°C) 63 °C, 15 min	Survival rate (%)‡	72 °C, 15 s	Survival rate (%)‡
Lim. fermentum Pom5	SA	9.36 ± 0.06^{A}	4.44 ± 0.12^{B}	47.48^{B}	5.80 ± 0.15^{B}	61.91^{B}
	SAGM	9.32 ± 0.06^{A}	7.22 ± 0.11^{A}	77.47^{A}	7.94 ± 0.05^{A}	85.18^{A}
	Non-encapsulated	9.31 ± 0.12^{A}	3.71 ± 0.18^{C}	39.90^{C}	4.49 ± 0.33^{C}	48.23^{C}
P. pentosaceus Chi8	SA	9.40 ± 0.04^{A}	4.65 ± 0.05^{B}	51.10^{B}	5.51 ± 0.17^{B}	58.70^{B}
	SAGM	9.36 ± 0.03^{A}	7.37 ± 0.14^{A}	78.75^{A}	6.33 ± 0.04^{A}	84.47^{A}
	Non-encapsulated	9.41 ± 0.05^{A}	2.21 ± 0.18^{C}	34.47^{C}	3.66 ± 0.12^{C}	50.51^{C}

Values represent the mean ± standard deviation of 3 determinations. Statistically significant differences within matrices are indicated by different capital letters (columns) at each pasteurization temperature, with a significance level of $p \leqslant 0.05$ (Tukey's test).

‡ Calculated by dividing the viable cells (cfu/g or cfu/ml) after pasteurization by the viable cells (cfu/g or cfu/ml) before pasteurization.

yield were determined, as these factors are crucial to consider for further evaluation of the encapsulated probiotics. The size of the microcapsules revealed that SAGM microbeads (2.26±0.01–2.29±0.01 mm) were slightly larger than SA microbeads (2.24±0.02–2.21±0.02 mm) but had a significantly higher encapsulation yield (96.64±0.81–98.97±0.73%). The larger size of SAGM microbeads may have provided a greater surface area for the interaction between the LAB strains and the hydrocolloid matrix, resulting in a more efficient entrapment of the cells and higher encapsulation yield. In contrast with previous studies, Prasanna and Charalampopoulos [17] reported that the size of SA microbeads (2.3±0.4 mm) was significantly smaller than that of SAGM microbeads (3.1±0.2 mm) but not significantly different in encapsulation yield. The incorporation of sodium alginate with goat milk in the microencapsulation process can have a significant

influence on the observed phenomenon. The combination of these 2 components brings together unique characteristics that positively impact the encapsulation process. Goat milk, with its proteins, fats, carbohydrates, and prebiotics, can interact with the sodium alginate matrix and the probiotic cells, resulting in improved entrapment and enhanced protection of the cells within the microbeads. This interaction has the potential to lead to higher yields of encapsulated probiotic cells. Regarding surface structure, SAGM microbeads exhibited a smoother wavy appearance with a denser and layered structure, which may have facilitated better interaction between the LAB strains and the hydrocolloid matrix, promoting a more efficient entrapment of the cells within the microbeads and potentially resulting in higher encapsulation. Similarly, Prasanna and Charalampopoulos [17] reported that SAGM microbeads displayed a crack-free surface, suggesting improved protection of microencapsulated cells against adverse conditions.

The survivability of LAB strains under GI conditions is a critical factor for ensuring their viability and functionality in the target site of the intestines. The results revealed that the free cells of the LAB strains were able to survive under artificial gastric juice conditions for up to 180 minutes, in contrast to the findings of Prasanna and Charalampopoulos [17], where free cells were not detectable up to 120 min. This discrepancy could potentially be attributed to the fact that the LAB strains used in this study were isolated from dog feces, which are known to be exposed to harsh conditions in the GI tract. These strains, having been exposed to such conditions, may have developed resilience and adaptation, leading to a longer survival time in the GI tract compared to LAB strains from other sources. In addition, microencapsulation with SAGM resulted in better protection for the cells compared to SA, which is consistent with previous studies that reported higher survival rates, likely attributed to the high buffer capacity of milk proteins that can protect LAB from the highly acidic environment [17, 30, 31]. These findings highlight the potential of SAGM microbeads as an effective delivery system for protecting probiotic LAB strains during passage through the GI tract.

The sequential impact of GI conditions on the survivability of LAB strains was a notable aspect investigated in this study. While many previous studies have examined the effects of individual GI conditions such as saliva, gastric, or intestinal juices, only a limited number of studies have investigated the sequential impact of multiple GI conditions. This study is one of the first of its kind to simulate sequential GI conditions, providing a more realistic representation of the complex environment of the GI tract. According to the results, SAGM microbeads showed the highest survival rate with viable cell counts remaining above 6 log cfu/g, which exceeds the recommended standards set by FDA for probiotic effectiveness in providing health benefits [32]. This indicates the potential of these microencapsulated LAB strains to provide health benefits as probiotics, even when subjected to the sequential challenges of the GI tract.

The study evaluated the release profiles of microencapsulated LAB strains under AIJ conditions using SA and SAGM matrices. Successful colonization of the GI tract is crucial for probiotics to effectively interact with the host and confer health benefits [33]. Previous studies have shown that microencapsulation-based methods can improve survival rates and retention of viable probiotics in reaching the colon [34–36]. In this study, special attention was given to timing the probiotic release from the microencapsulated matrix to provide a sufficient number of viable probiotics during the estimated transit time in the large bowel, which is reported to range from 427 to 2,573 min in dogs [37].

Maintaining the viability of probiotics during storage is an important consideration for their commercial applications, as probiotic products are often stored under refrigeration to prolong their shelf life. In this study, goat milk was chosen as the carrier for probiotics because it is a common ingredient in pet food and is known to be easily digestible when compared to cow milk [38]. Both free and microencapsulated LAB strains, using both SA and SAGM matrices, were able to maintain viability levels exceeding 6 log cfu/g in goat milk during storage at 4 °C for 28 days. SAGM capsules provided the best protection for cells during 28 days of storage, although cell survival may vary depending on the strain used. Similar findings have been reported in other studies where alginate-based microbeads provided effective protection for probiotics during refrigerated storage. For instance, encapsulation of *L. gasseri* and *B. bifidum* in chitosan-coated alginate microspheres was shown to maintain viability throughout a 28-day storage period at 4 °C [21]. Additionally, Prasanna and Charalampopoulos [17] demonstrated that alginate-dairy microbeads offered better protection for cells during refrigerated storage compared to SACH (SA/casein hydrolysate) and SA. This result suggests that these probiotic strains can be incorporated with goat milk both as an encapsulation material and as a colloid for suspending both encapsulated and un-encapsulated probiotic cells, leading to the development of feed products for dogs without significant loss of viability, potentially conferring health benefits.

The survivability of microencapsulated LAB strains under pasteurization temperature was evaluated in the study, as pasteurization is a common heat treatment used in the food industry to ensure food safety by reducing pathogenic microorganisms [39]. However, the minimal reduction in probiotic viability during the heating process has been a challenge for research and

development [40]. The study found that microencapsulated LAB strains using SAGM demonstrated efficacy in enhancing the thermal stability of both *Lim. fermentum* Pom5 and *P. pentosaceus* Chi8 when compared to SA, suggesting that the SAGM matrix provided better protection for the bacterial cells against pasteurization heat treatment. The milk proteins surrounding the bacterial cells as well as the thicker structure of SAGM may contribute to the improved heat stability of the bacteria by slowing the diffusion of heat [41]. The use of microencapsulation, particularly with SAGM as a matrix, in this study may offer a potential solution to improve the thermal stability of probiotics during pasteurization, which is a critical step in ensuring the food safety of various food and beverage products.

All findings of the study suggest that SAGM microbeads may offer improved encapsulation yields, survivability in artificial GI conditions, and viability during refrigerated storage compared to SA microbeads. The use of sequential GI conditions in the study provides a more realistic representation of the *in vivo* environment, and the survivability of LAB strains may be influenced by their source of origin, as seen in the resilience of LAB strains isolated from dog feces. These findings contribute to the understanding of the potential benefits of SAGM microbeads for probiotic delivery and highlight the importance of considering various factors in designing effective probiotic delivery systems.

CONCLUSION

The encapsulation of alginate with goat milk proved to be the most effective method for protecting the viability of *Lim. fermentum* Pom and *P. pentosaceus* Chi8 in various conditions, including artificial GI conditions, refrigeration at 4 °C for 28 days, and pasteurization. Furthermore, the incorporation of non-encapsulated and microencapsulated bacterial cells in goat milk as a delivery system for probiotics to dogs showed improved survival of probiotic bacteria and extended storage stability. This alternative approach holds promise for maintaining the viability of probiotics during storage for longer periods of time.

Acknowledgements: This work was supported by the Srinakharinwirot University Fund (grant number 653/2563) and Graduate Study Development Grant from National Research Council of Thailand (NRCT) (grant number N41A660291).

REFERENCES

1. Suchodolski JS, Simpson K (2013) Canine gastrointestinal microbiome in health and disease. *Vet Focu* **23**, 22–28.
2. Sharma S, Chaubey KK, Singh SV, Gupta S (2022) Symbiotic microbiota: A class of potent immunomodulators. *ScienceAsia* **48**, 855–865.
3. Guard BC, Mila H, Steiner JM, Mariani C, Suchodolski JS (2017) Characterization of the fecal microbiome during neonatal and early pediatric development in puppies. *PLoS One* **17**, e0175718.
4. Schmitz S, Suchodolski J (2016) Understanding the canine intestinal microbiota and its modification by pro-, pre-, and synbiotics – what is the evidence? *Vet Med Sci* **11**, 71–94.
5. FAO/WHO (2002) *Guidelines for the Evaluation of Probiotics in Food*. Food and Agriculture Organization of the United Nations, World Health Organization, London, Ontario. Available at: http://www.who.int/foodsafety/fs_management/en/probiotic_guidelines.pdf.
6. Macpherson AJ, Uhr T (2004) Induction of protective IgA by intestinal dendritic cells carrying commensal bacteria. *Science* **12**, 1662–1665.
7. Narvhus JA, Axelsson L (2003) Lactic acid bacteria. In: Caballero B (ed) *Encyclopedia of Food Sciences and Nutrition*, 2nd edn, Academic Press, Oxford, pp 3465–3472.
8. Leroy F, De Vuyst L (2004) Lactic acid bacteria as functional starter cultures for the food fermentation industry. *Food Sci Technol* **15**, 67–78.
9. Piątek J, Gibas-Dorna M, Olejnik A, Krauss H, Wierzbicki K, Żukiewicz-Sobczak W, Głowacki M (2012) The viability and intestinal epithelial cell adhesion of probiotic strain combination – *in vitro* study. *Ann Agric Environ Med* **19**, 99–102.
10. Bhat AR, Irorere VU, Bartlett T, Hill D, Kedia G, Charalampopoulos D, Nualkaekul S, Radecka I (2015) Improving survival of probiotic bacteria using bacterial poly-γ-glutamic acid. *Int J Food Microbiol* **196**, 24–31.
11. Moumita S, Goderska K, Johnson EM, Das B, Indira D, Yadav R, Jayabalan R (2017) Evaluation of the viability of free and encapsulated lactic acid bacteria using *in vitro* gastrointestinal model and survivability studies of synbiotic microcapsules in dry food matrix during storage. *LWT Food Sci Technol* **77**, 460–467.
12. Sultana K, Godward G, Reynolds N, Arumugaswamy R, Peiris P, Kailasapathy K (2000) Encapsulation of probiotic bacteria with alginate-starch and evaluation of survival in simulated gastrointestinal conditions and in yoghurt. *Int J Food Microbiol* **62**, 47–55.
13. Shi LE, Li ZH, Zhang ZL, Zhang TT, Yu WM, Zhou ML, Tang ZX (2013) Encapsulation of *Lactobacillus bulgaricus* in carrageenan-locust bean gum coated milk microspheres with double layer structure. *LWT Food Sci Technol* **54**, 147–151.
14. Rajam R, Karthik P, Parthasarathi S, Joseph G, Anandharamakrishnan C (2012) Effect of whey protein-alginate wall systems on survival of microencapsulated *Lactobacillus plantarum* in simulated gastrointestinal conditions. *J Funct Foods* **4**, 891–898.
15. Cook MT, Tzortzis G, Khutoryanskiy VV, Charalampopoulos D (2013) Layer-by-layer coating of alginate matrices with chitosan-alginate for the improved survival and targeted delivery of probiotic bacteria after oral administration. *J Mater Chem B* **1**, 52–60.
16. Razavi S, Janfaza S, Tasnim N, Gibson DL, Hoorfar M (2021) Microencapsulating polymers for probiotics delivery systems: Preparation, characterization, and applications. *Food Hydrocoll* **120**, 106882.
17. Prasanna PHP, Charalampopoulos D (2018) Encapsulation of *Bifidobacterium longum* in alginate-dairy matrices and survival in simulated gastrointestinal conditions, refrigeration, cow milk and goat milk. *Food Biosci* **21**,

72–79.
18. Maciel GM, Chaves KS, Grosso CRF, Gigante ML (2014) Microencapsulation of *Lactobacillus acidophilus* La-5 by spray-drying using sweet whey and skim milk as encapsulating materials. *J Dairy Sci* **97**, 1991–1998.
19. Silanikove N, Leitner G, Merin U (2015) The Interrelationships between lactose intolerance and the modern dairy industry: Global perspectives in evolutional and historical backgrounds. *Nutrients* **7**, 7312–7331.
20. Foongsawat N, Sunthornthummas S, Nantavisai K, Surachat K, Rangsiruji A, Sarawaneeyaruk S, Insian K, Sukontasing S, et al (2023) Isolation, characterization, and comparative genomics of the novel potential probiotics from canine feces. *Food Sci Anim Resour* **43**, 685–702.
21. Bao S-S, Hu X-C, Zhang K, Xu X-K, Zhang H-M, Huang H (2010) Characterization of spray-dried microalgal oil encapsulated in cross-linked sodium caseinate matrix induced by microbial transglutaminase. *J Food Sci* **76**, E112–E118.
22. Sun W, Griffiths MW (2000) Survival of bifidobacteria in yogurt and simulated gastric juice following immobilization in gellan-xanthan beads. *Int J Food Microbiol* **61**, 17–25.
23. Chávarri M, Marañón I, Ares R, Ibáñez FC, Marzo F, del Carmen Villarán M (2010) Microencapsulation of a probiotic and prebiotic in alginate-chitosan capsules improves survival in simulated gastro-intestinal conditions. *Int J Food Microbiol* **142**, 185–189.
24. Fareez IM, Lim SM, Mishra RK, Ramasamy K (2015) Chitosan coated alginate-xanthan gum bead enhanced pH and thermotolerance of *Lactobacillus plantarum* LAB12. *Int J Biol Macromol* **72**, 1419–1428.
25. Mao L, Pan Q, Yuan F, Gao Y (2019) Formation of soy protein isolate-carrageenan complex coacervates for improved viability of *Bifidobacterium longum* during pasteurization and *in vitro* digestion. *Food Chem* **276**, 307–314.
26. Deschamps C, Humbert D, Zentek J, Denis S, Priymenko N, Apper E, Blanquet-Diot S (2022) From Chihuahua to Saint-Bernard: How did digestion and microbiota evolve with dog size. *Int J Biol Sci* **18**, 5086–5102.
27. Manninen TJ, Rinkinen ML, Beasley SS, Saris PE (2006) Alteration of the canine small-intestinal lactic acid bacterium microbiota by feeding of potential probiotics. *Appl Environ Microbiol* **72**, 6539–6543.
28. Melo CCS, Freire AS, Galdeano MA, Costa CF, Gonçalves APDO, Dias FS, Menezes DR (2021) Probiotic potential of *Enterococcus hirae* in goat milk and its survival in canine gastrointestinal conditions simulated *in vitro*. *Res Vet Sci* **138**, 188–195.
29. Strompfov V, Marcinakov M, Simonova M, Bogovic-Matijasic B, Laukova A (2006) Application of potential probiotic *Lactobacillus fermentum* AD1 strain in healthy dogs. *Anaerobe* **12**, 75–79.
30. Krasaekoopt W, Bhandari B, Deeth H (2004) The influence of coating materials on some properties of alginate beads and survivability of microencapsulated probiotic bacteria. *Int Dairy J* **14**, 737–743.
31. Guérin D, Vuillemard JC, Subirade M (2003) Protection of bifidobacteria encapsulated in polysaccharide-protein gel beads against gastric juice and bile. *J Food Prot* **66**, 2076–2084.
32. FDA (2003) Guidelines for industry. Early clinical trials with live biotherapeutic products: chemistry, manufacturing, and control information. Available at: https://www.fda.gov/downloads/Biologi.../UCM292704.pdf.
33. Alp D, Kuleasan H (2019) Adhesion mechanisms of lactic acid bacteria: Conventional and novel approaches for testing. *World J Microbiol Biotechnol* **35**, 1–9.
34. Han S, Lu Y, Xie J, Fei Y, Zheng G, Wang Z, Liu J, Lv L, et al (2021) Probiotic gastrointestinal transit and colonization after oral administration: A long journey. *Front Cell Infect Microbiol* **11**, 609722.
35. Martin MJ, Lara-Villoslada F, Ruiz MA, Morales ME (2015) Microencapsulation of bacteria: A review of different technologies and their impact on the probiotic effects. *Innovative Food Sci Emerg Technol* **27**, 15–25.
36. Yao MF, Wu J, Li B, Xiao H, McClements DJ, Li LJ (2017) Microencapsulation of *Lactobacillus salivarious* Li01 for enhanced storage viability and targeted delivery to gut microbiota. *Food Hydrocoll* **72**, 228–236.
37. Boillat S, Gaschen FP, Hosgood GL (2010) Assessment of the relationship between body weight and gastrointestinal transit times measured by use of a wireless motility capsule system in dogs. *Am J Vet Res* **71**, 898–902.
38. Paszczyk B, Czarnowska-Kujawska M, Klepacka J, Tónska E (2023) Health-promoting ingredients in goat's milk and fermented goat's milk drinks. *Animals* **13**, 907.
39. Li X, Xu X, Wang L, Regenstein JM (2019) Effect of ohmic heating on physicochemical properties and the key enzymes of water chestnut juice. *J Food Process Preserv* **3**, e13919.
40. Capra ML, Quiberoni A, Reinheimer JA (2004) Thermal and chemical resistance of *Lactobacillus casei* and *Lactobacillus paracasei* bacteriophages. *Lett Appl Microbiol* **38**, 499–504.
41. Mousazadeh M, Mousavi M, Askari G, Kiani H, Adt I, Gharsallaoui A (2018) Thermodynamic and physiochemical insights into chickpea Protein-Persian gum interactions and environmental effects. *Int J Biol Macromol* **119**, 1052–1058.