www.fagr.bu.edu.eg

Annals of Agricultural Science, Moshtohor (ASSJM)
https://assjm.journals.ekb.eg/

© 2023, Faculty of Agriculture, Benha University, Egypt. ISSN:1110-0419

Original Article Vol. 63(1) (2025), 149 – 162 DOI: 10.21608/assjm.2025.354402.1345

Investigation of Encapsulated Lactic Acid Bacteria under Simulated Gastrointestinal Conditions

Esraa E. Aboofa, Mohamed B. El-Alfy, Abd El-Aty M. Abdelaty, Atallah A. Atallah
Dairy Sci. Dept., Fac. Agric., Moshtohor, Benha University, Egypt.
Corresponding author: esraa.mohamed@fagr.bu.edu.eg

Abstract

The main goal of this research was to examine the viability of five strains of encapsulated lactic acid bacteria [LAB] (*Lactobacillus plantarum* ATCC14917, *Lactobacillus casei* NCAIM B01137, *Lactobacillus rhamnosus* ISO7, *Bifidobacterium breve* ISO8 *and Leuconostoc mesenteroides* subsp. *mesenteroides* DSM 20343) in model solutions designed to simulate the acidity, bile salt, and phenol concentrations identified within the human gastrointestinal system. The capsules were formulated using sodium alginate and kappa (k)-carrageenan. The application of sodium alginate and k-carrageenan has been shown to provide ideal properties for the encapsulation of live bacteria strains. All bacteria grew well in the presence of 0.1 and 0.5% bile salt during incubation to 6 hours except *Leuconostoc mesenteroides* subsp. *mesenteroides* DSM 20343 was sensitive to bile salt concentrations. At pH 7.0 (control), survival of LAB in MRS medium remained stable after 6 hours of incubation at 37℃, regardless of whether they were encapsulated or non-encapsulated. At a pH of 3, the number of encapsulated LAB exhibited a minor reduction after 6 hours of incubation, whereas the free cells showed a one-log decrease under comparable conditions. The survival of all LAB strains decreased by increasing the salt concentration during the incubation time up to 6 hours. All tested LAB strains showed resistance to Gentamycin, Kanamycin, Erythromycin and Lincomycin. Research indicated that capsules made from sodium alginate and k-carrageenan effectively safeguarded the tested strains against gastric acid, leading to a significant release of viable bacteria in the intestine.

Keywords: Encapsulation; Lactic acid bacteria; Sodium alginate; Kappa (k)-Carrageenan; viability

Introduction

Over the past two decades, interest in the significance of probiotic bacteria for human health has grown. Lactic acid bacteria are recognized as the most advantageous microorganisms found in the intestinal tract. Consequently, the dairy industry has concentrated on integrating these beneficial bacteria into dairy products, resulting in the development of a new category of "probiotic health" dairy and foods. The International Dairy Federation (IDF) states that an effective probiotic should contain a minimum of 10^7 CFU/g at the time of use. Therefore, **Mahmoud *et al.* (2020)** suggested that a minimum dosage of probiotic bacteria of no less than 10^6 CFU/g should be consumed to achieve positive health benefits. Similarly, **Abd El-Salam and El-Shibiny, (2015)** asserted that a probiotic product must contain at least 1×10^9 CFU/g. Nevertheless, research has shown that the bacteria may not remain viable in adequate quantities when added to dairy products and throughout their transit in the gastrointestinal tract (**Shah and Lankaputhra, 2002**). The strategy of equipping probiotic living cells with a protective barrier to shield them from unfavorable environmental conditions is currently garnering significant attention (**Kailasapathy, 2002**). Among the various methods for immobilizing living cells, the use of calcium alginate beads has been commonly employed for the immobilization of LAB (**Sheu and Marshall, 1993**). Alginate is particularly beneficial due to its non-toxic properties concerning the immobilized cells, and it is recognized as a safe food additive (**Prevost and Divies, 1992**). Furthermore, the capacity for reversible encapsulation, achieved through the solubilization of alginate gel via the removal of calcium ions, along with the possibility of releasing the encapsulated cells within the human gastrointestinal tract, constitutes an additional advantage (**Chandramouli *et al.*, 2004**).

Microencapsulation is a method that effectively safeguards sensitive microorganisms, such as LAB strains, from losing cell viability during gastrointestinal transit by encapsulating them within a biopolymeric matrix (**Soukoulis *et al.*, 2013**). As protection from external damaging factors, in microencapsulation, the semipermeable membrane that surrounds the liquid core is required to preserve cell viability during processing and storage (**Mu *et***

***al.*, 2018** and **Pradipta *et al.*, 2019**). The primary characteristics of microencapsulation in relation to probiotics include its effective protection against acidic environments and the careful execution of the preparation process, which ensures the integrity of the encapsulated cells (**Burgain *et al.*, 2011**). It is essential that the chosen polymer is non-cytotoxic and free from antimicrobial properties. Furthermore, when applied in the food industry, especially concerning probiotics, the material must be edible and able to form a barrier that protects the encapsulated content. In this study, *in vitro* experiments utilizing encapsulated LAB were performed to determine the protective function of capsules in facilitating the effective transit of live bacteria through the gastrointestinal system. Five strains of LAB (*Lactobacillus plantarum* ATCC14917, *Lactobacillus casei* NCAIM B01137, *Lactobacillus rhamnosus* ISO7, *Bifidobacterium breve* ISO8 *and Leuconostoc mesenteroides* subsp. *mesenteroides* DSM 20343) underwent in vitro analysis to assess their probiotic potential.

Materials and Methods

2.1. Materials:

Sodium alginate, k-carrageenan, bile salt, and phenol were acquired from El-Gomhoria Company in Cairo, Egypt. A commercially pure fine-grade NaCl was sourced from El-Naser Company, Egypt. The $CaCl_2$ was procured from Sigma Chemical Company, Egypt. MRS medium was obtained from Future Company, Egypt. Fresh milk from cows and buffaloes was collected from the herds at Moshtohor, Faculty of Agriculture, Benha University.

2.2. Lactic acid bacteria:

Five strains (*Lactobacillus* (Lb.) *plantarum* ATCC14917, Lb. *casei* NCAIM B01137, Lb. *rhamnosus* ISO7, *Bifidobacterium breve* ISO8 *and Leuconostoc mesenteroides ssp mesenteroides* DSM 20343) were kindly acquired from the Institute of Microbiology at the Federal Research Center for Nutrition and Food in Kiel, Germany, through personal communication with Dr. El-Sayed Ismail from the Dairy Science Department at the Faculty of Agriculture, Moshtohor Benha University, Egypt.

2. Methods:

2.1. Bacterial Cultivation:

The LAB cells were cultivated in MRS medium broth at a temperature of 37 ºC for a duration of 24 hours. The strains underwent reactivation through two successive transfers in the same media, achieving a concentration of 10^7 CFU/ml, and were subsequently stored in a refrigerator for use within 24 hours. Following the growth period, the cells were isolated *via* centrifugation for 10 minutes at 6000 rpm and 4 ºC. The supernatant was discarded, and the cell pellet was washed twice with cold (4 ºC), sterilized physiological saline solution (0.1%), resulting in an approximate concentration of 6×10^{10} CFU/ml. All steps of immobilization were performed under aseptic conditions using sterilized solutions, which were treated at 121 ºC for 15 minutes.

2.2. Preparation of Encapsulated lactic acid bacteria:

2.2.1. Preparation of alginate gel beads:

Gel beads for reservoir-type capsules were created by suspending 20 grams of cells (wet weight) in 1000 milliliters of a sterile 2% sodium alginate solution. This mixture was then introduced dropwise using a micropipette into a flask containing a sterile 2% (w/v) $CaCl_2$ solution while being agitated at 30 rpm. Gel beads with an approximate diameter of 4 mm were formed and allowed to remain in the $CaCl_2$ solution for 60 minutes to facilitate the hardening of the gel. Subsequently, the gel beads were rinsed with a sterile 0.1% physiological saline solution to eliminate any excess calcium ions and entrapped cells **(Kebary *et al.*, 1998).**

2.2.2. Preparation of k-carrageenan gel beads:

Gel beads of reservoir-type microcapsules were created by suspending 20 g of cells (wet weight) in 1000 ml of a sterile 3% k-carrageenan solution. This mixture was then added dropwise with a micropipette into a flask containing a sterile 3% (w/v) KCl_2 solution while being agitated at 20 rpm. The gel beads were allowed to remain in the KCl_2 solution for 60 minutes to facilitate the hardening of the gel. Subsequently, the gel beads were washed with a sterile 0.1% physiological saline solution to eliminate any excess potassium ions and trapped cells (**Dinakar and Mistry, 1994**).

2.3. Acid tolerance:

The assessment of acid tolerance was conducted by cultivating the examined LAB in MRS broth **(Lin and Chen, 2000).** Viable cell counts were determined on MRS agar at various times (0, 2, 4 and 6 h) of incubation at 37 ºC. The plates were maintained at a temperature of 37 ºC for a duration of 72 hours in an anaerobic environment.

2.4. Bile salts resistance:

The bile tolerance of the evaluated LAB strains to bile salts was assessed using MRS broth with varying concentrations (0.0, 0.1, 0.2, 0.3, 0.5, and 1.0 % w/v) of ox gall bile salts. Viable cell counts were measured on MRS agar at different incubation times (0, 2, 4, and 6 h) at a temperature of 37 ºC. The plates were incubated anaerobically at 37 ºC for a duration of 72 hours (**Kociubinski *et al.*, 1999)**.

2.5. Phenol resistance:

Phenol resistance of the tested LAB strains was investigated as described by **Suskovic *et al.* (1997).** The survival of LAB strains was determined on MRS broth with varying concentrations (0.0, 0.1, 0.2, 0.3, 0.4 and 0.5% w/v) of phenol and viable cell counts were measured on MRS agar at various times (0, 2, 4 and 6 h) at a temperature of 37 ºC. The plates were maintained in an anaerobic environment at a temperature of 37 ºC for a duration of 72 hours.

2.6. Antibiotic susceptibility:

LAB strains were tested for antibiotic resistance against six selected antibiotics, *i.e*. (Ampicillin, Cefotaxime, Gentamycin, Kanamycin, Erythromycin and Lincomycin) according to the method of **Basyigit *et al.* (2006)**.

2.7. Effect of sodium chloride (NaCl) concentrations:

The effect of NaCl concentrations of different LAB strains was done according to the method described by **Sabikhi *et al*. (2010).**

Results and Discussions

Bile salt tolerance:

It is crucial that when consuming dairy or food products that have passed through the acidic environment of the stomach, the application of LAB strains must ensure their survival in the presence of bile salts in the intestine.

Table (1) shows the survival of the non-encapsulated and the encapsulated LAB after 6 hours of different concentration (0.0, 0.1, 0.2, 0.3, 0.5 and 1.0 %) of ox-gall bile salts. The obtained results appear that the survival of LAB for the various carrier materials (sodium alginate and k-carrageenan) showed no difference during incubation time, the findings from the current study indicate that the protective effect of encapsulating LAB with alginate and carrageenan was documented, along with the specific strains of tested bacteria and the unfavorable conditions they faced. Notably, the study revealed that LAB encapsulated in alginate and carrageenan demonstrated significantly greater survival rates compared to free cells when subjected to bile salt concentrations of 0.5% and 1.0% over a duration of 6 hours. All strains were grown well in the presence of 0.1 and 0.5% bile salt during incubation to 6 hours except *Leuconostoc mesenteroides* ssp *mesenteroides* was sensitive to bile salt concentrations. Overall, the viability of both non-encapsulated and encapsulated LAB diminished as the duration of exposure increased. Nevertheless, the survival rates of the different encapsulated LAB throughout the exposure period were observed to be superior to those of the free cells suspended in a 1% solution, regardless of the presence of additional carrier materials. This clearly indicates that encapsulation with the various tested carrier materials protected LAB cells against bile salts. After six hours of exposure, the survival rate of encapsulated LAB surpassed that of the free cells. **(Table 1**).

A similar finding was revealed by **Azam *et al.* (2021**), who noted that free cells could not survive in the bile salt solution after 2 hours of incubation. In contrast, all encapsulated formulations maintained a viable cell count exceeding 6 log CFU/ml, indicating that the encapsulation of probiotics offers protection to free cells. **Mahmoud *et al*. (2022)** reported similar results, noting that all isolates demonstrated significant growth in the presence of 0.5% bile salts during a 4-hour incubation period, except for *Lactococcus* (Lc). *lactis* ASO 26, which exhibited a decreasing growth trend throughout the incubation. The strains *Lb. delbrueckii* ASO100, *Lb. paracasei* ASO32, and *Lb. plantarum* ASO50 showed the highest levels of tolerance to bile salts, respectively. Additionally, **Abedfar *et al.* (2021)** indicated that conditions mimicking the gastrointestinal tract revealed that bile salt solutions had a diminished impact on the viability of the *Lb. plantarum* population in both exopolysaccharide-encapsulated and control samples when compared to free cells. This phenomenon may be attributed to bile salts compromising cell wall integrity, leading to the observed detrimental effects on certain probiotic bacteria (**Shi *et al*., 2016**). A similar pattern of results was previously documented by **Atallah (2013); Pupa *et al.* (2021); Ali *et al.* (2023) and Dumitru *et al.* (2023).**

Table 1. Survival (Log CFU ml $^{-1}$) of non-encapsulated and encapsulated lactic acid bacteria in the presence of bile salt concentrations up to 6 hours at 37°C in MRS medium.

Strains	Materials	Bile salt (%)	Incubation time (hours)			
			Zero	2	4	6
Lb. plantarum ATCC14917	Non-capsulated	Zero	8.67	8.70	8.74	**8.79**
	Sodium alginate		8.69	8.69	8.75	**8.77**
	K-carrageenan		8.57	8.62	8.67	**8.76**
	Non-capsulated	0.1	8.60	8.68	8.70	**8.76**
	Sodium alginate		8.67	8.68	8.7	**8.71**
	K-carrageenan		8.53	8.51	8.39	**8.29**
	Non-capsulated	0.2	8.66	8.58	8.58	**8.50**
	Sodium alginate		8.66	8.58	8.57	**8.47**
	K-carrageenan		8.55	8.51	8.47	**8.18**
	Non-capsulated	0.3	8.65	8.58	8.32	**8.24**
	Sodium alginate		8.66	8.57	8.34	**8.25**
	K-carrageenan		8.54	8.49	8.42	**8.18**
	Non-capsulated	0.5	8.67	8.51	8.32	**8.13**
	Sodium alginate		8.66	8.54	8.19	**8.14**

	K-carrageenan		8.53	8.47	8.25	**8.08**
	Non-capsulated	1.0	8.66	8.43	8.44	**7.74**
	Sodium alginate		8.67	8.28	8.83	**7.53**
	K-carrageenan		8.54	8.37	8.07	**7.93**
Lb. casei NCAIM B01137	Non-capsulated	Zero	8.65	8.64	8.66	**8.78**
	Sodium alginate		8.54	8.38	8.54	**8.59**
	K-carrageenan		8.39	8.29	8.34	**8.59**
	Non-capsulated	0.1	8.61	8.55	8.64	**8.68**
	Sodium alginate		8.49	8.36	8.30	**8.71**
	K-carrageenan		8.32	8.27	8.17	**5.94**
	Non-capsulated	0.2	8.63	8.49	8.45	**8.02**
	Sodium alginate		8.54	8.29	7.95	**8.47**
	K-carrageenan		8	8.26	7.75	**5.91**
	Non-capsulated	0.3	8.60	8.39	8.18	**7.48**
	Sodium alginate		8.39	8.17	7.86	**8.25**
	K-carrageenan		7.99	8.03	7.69	**5.90**
	Non-capsulated	0.5	7.86	8.31	8	**7.50**
	Sodium alginate		8.04	8.14	7.77	**8.14**
	K-carrageenan		7.02	7.98	7.70	**5.91**
	Non-capsulated	1.0	7.27	7.79	7.97	**7.64**
	Sodium alginate		7.68	7.49	7.47	**7.53**
	K-carrageenan		6.50	7.47	7.30	**5.90**
Lb. rhamnosus ISO7	Non-capsulated	Zero	8.67	8.71	8.75	**8.79**
	Sodium alginate		8.69	8.72	8.75	**8.77**
	K-carrageenan		8.61	8.68	8.71	**8.67**
	Non-capsulated	0.1	8.7	8.69	8.72	**8.76**
	Sodium alginate		8.68	8.69	8.73	**8.76**
	K-carrageenan		8.62	8.67	8.69	**8.66**
	Non-capsulated	0.2	8.68	8.69	8.65	**8.56**
	Sodium alginate		8.69	8.7	8.72	**8.6**
	K-carrageenan		8.61	8.57	8.51	**8.49**
	Non-capsulated	0.3	8.67	8.63	8.32	**8.22**
	Sodium alginate		8.68	8.69	8.32	**8.21**
	K-carrageenan		8.62	8.56	8.29	**8.17**
	Non-capsulated	0.5	8.66	8.55	8.27	**8.11**
	Sodium alginate		8.67	8.58	8.29	**8.11**
	K-carrageenan		8.61	8.56	8.21	**8.07**
	Non-capsulated	1.0	8.66	8.56	8.15	**7.42**
	Sodium alginate		8.67	8.53	8.24	**7.08**
	K-carrageenan		8.58	8.43	7.78	**7.07**
Bifidobacterium breve ISO8	Non-capsulated	Zero	8.62	8.76	8.78	**8.81**
	Sodium alginate		8.64	8.75	8.77	**8.79**
	K-carrageenan		8.58	8.62	8.67	**8.71**
	Non-capsulated	0.1	8.62	8.33	7.91	**7.65**
	Sodium alginate		8.63	8.33	7.91	**7.61**
	K-carrageenan		8.57	8.29	7.84	**7.54**
	Non-capsulated	0.2	8.63	8.28	7.9	**7.58**
	Sodium alginate		8.63	8.29	7.91	**7.56**
	K-carrageenan		8.57	7.97	7.79	**7.49**
	Non-capsulated	0.3	8.62	8.28	7.79	**7.2**
	Sodium alginate		8.62	8.28	7.84	**7.38**
	K-carrageenan		8.55	7.95	7.63	**7.2**
	Non-capsulated	0.5	8.62	8.26	7.74	**7.05**
	Sodium alginate		8.62	8.25	7.75	**7.04**
	K-carrageenan		8.57	7.9	7.36	**6.95**
	Non-capsulated	1.0	8.61	8.2	7.6	**6.77**
	Sodium alginate		8.62	8.21	7.6	**6.69**
	K-carrageenan		8.5	7.77	7.44	**6.63**
Leuconostoc mesenteroides	Non-capsulated	Zero	8.2	8.45	8.72	**8.45**
	Sodium alginate		8.24	8.70	8.69	**8.78**

subsp. *mesenteroides* DSM 20343	K-carrageenan		7.56	8.30	8.54	**8.79**
	Non-capsulated	0.1	7.33	6.54	6.28	**6.00**
	Sodium alginate		7.09	6.49	6.29	**6.12**
	K-carrageenan		7.57	6.32	6.09	**5.94**
	Non-capsulated	0.2	6.53	6.25	6.17	**6.01**
	Sodium alginate		6.84	6.28	6.17	**6.05**
	K-carrageenan		7.58	6.30	6.02	**5.91**
	Non-capsulated	0.3	6.53	6.25	6.12	**6.04**
	Sodium alginate		6.83	6.28	6.9	**6.01**
	K-carrageenan		6.21	6.14	6.01	**5.90**
	Non-capsulated	0.5	6.38	6.16	6.21	**6.00**
	Sodium alginate		6.50	6.17	6.3	**5.98**
	K-carrageenan		6.19	6.11	6.01	**5.91**
	Non-capsulated	1.0	6.33	6.7	5.99	**5.70**
	Sodium alginate		6.47	6.08	5.99	**5.68**
	K-carrageenan		6.18	6.01	5.73	**5.90**

Acid tolerance:

The data obtained demonstrated that the survival of the tested bacteria was not great by increasing the pH of the environment from 3.0 to 8.0 for 6 hours (**Table 2**). The survival of all tested bacteria remained higher than 10^7 CFU ml^{-1} during incubation time (6 h) at 37℃. At a pH level of 7.0 (control), viability of LAB in MRS medium was consistent after 6 hours of incubation at 37℃, regardless of whether the cells were encapsulated or non-encapsulated. At a pH of 6, the LAB counts remained stable for both free and encapsulated forms throughout the 6-hour incubation period at 37℃. However, at pH 3, there was a minor reduction in the number of encapsulated LAB after 6 hours, while free cells experienced a more significant decline by one log cycle under the same conditions. At pH 8.0, a slight decrease in the number of strains was observed in both free and encapsulated states over the 6-hour incubation at 37℃. Conversely, the survival rate of encapsulated LAB, whether using sodium alginate or k-carrageenan, remained stable throughout the entire exposure duration. Consequently, the encapsulated LAB demonstrated superior survival compared to their non-encapsulated counterparts across varying pH levels. This study indicates that encapsulation with sodium alginate and k-carrageenan can effectively enhance the survival of LAB in different pH environments.

LABs were subjected to pH levels of 3.0 and 2.0 during a growth period of 3 hours at 37 ◦C. The viability rates of several of the 14 isolates were found to be greater at pH 3.0 than at pH 2.0. Additionally, following the 3-hour incubation, the isolates experienced a reduction in survivability due to exposure to both pH levels, with decreases ranging from 9% to 100%. At pH 3.0, survivability was compared to an environment with neutral pH (6.5 ± 0.2). Notably, after 3 hours at pH 2.0, there was a significant drop in the survival rates of eight isolates, with no substantial difference observed in the number of viable cells (**Dumitru *et al.*, 2023**). A similar pattern of results was previously documented by **Pupa *et al.* (2021); Mahmoud *et al.* (2022) and Ali *et al.* (2023).**

Table 2. Viability (Log CFU ml^{-1}) of non-encapsulated and encapsulated lactic acid bacteria up to 6 hours of exposure to different pH values at 37℃ in MRS medium.

Strains	Materials	pH values	Incubation time (hours)			
			Zero	2	4	6
Lb. plantarum ATCC14917	Non-capsulated	3	8.45	8.33	8.2	**8.2**
	Sodium alginate		8.44	8.39	8.23	**8.18**
	K-carrageenan		8.27	8.23	8.21	**8.14**
	Non-capsulated	5	8.47	8.34	8.27	**8.18**
	Sodium alginate		8.46	8.33	8.29	**8.21**
	K-carrageenan		8.25	8.21	8.16	**8.08**
	Non-capsulated	6	8.43	8.43	8.68	**8.77**
	Sodium alginate		8.47	8.44	8.69	**8.76**
	K-carrageenan		8.26	8.29	8.34	**8.4**
	Non-capsulated	7	8.46	8.42	8.7	**8.76**
	Sodium alginate		8.43	8.44	8.7	**8.78**
	K-carrageenan		8.26	8.32	8.38	**8.55**
	Non-capsulated	8	8.45	8.35	8.38	**8.07**
	Sodium alginate		8.46	8.39	8.36	**8.07**

	K-carrageenan		8.24	8.34	8.37	**8.04**
Lb. casei NCAIM B01137	Non-capsulated	3	8.72	8.34	7.63	**7.47**
	Sodium alginate		8.32	7.79	7.60	**7.23**
	K-carrageenan		8.67	8.44	8.23	**7.20**
	Non-capsulated	5	8.07	8.48	8.62	**8.77**
	Sodium alginate		8.26	8.43	8.50	**8.76**
	K-carrageenan		8.16	8.03	7.92	**8.75**
	Non-capsulated	6	7.97	8.55	8.60	**8.76**
	Sodium alginate		7.85	8.53	8.56	**8.75**
	K-carrageenan		7.62	7.95	7.86	**8.75**
	Non-capsulated	7	7.87	8.35	7.72	**7.30**
	Sodium alginate		8.07	8.43	7.87	**7.69**
	K-carrageenan		7.89	8.31	7.70	**7.73**
	Non-capsulated	8	8.12	7.83	7.65	**7.30**
	Sodium alginate		8.14	8	8.09	**7.95**
	K-carrageenan		7.98	7.91	7.69	**7.30**
Lb. rhamnosus ISO7	Non-capsulated	3	8.43	8.38	8.3	**8.25**
	Sodium alginate		8.43	8.37	8.29	**8.26**
	K-carrageenan		8.42	8.34	8.16	**8.04**
	Non-capsulated	5	8.64	8.37	8.28	**8.21**
	Sodium alginate		8.62	8.36	8.27	**8.2**
	K-carrageenan		8.39	8.32	8.26	**8.09**
	Non-capsulated	6	8.47	8.45	8.67	**8.79**
	Sodium alginate		8.45	8.44	8.66	**8.8**
	K-carrageenan		8.39	8.39	8.59	**8.53**
	Non-capsulated	7	8.46	8.43	8.72	**8.79**
	Sodium alginate		8.46	8.44	8.71	**8.79**
	K-carrageenan		8.41	8.39	8.67	**8.71**
	Non-capsulated	8	8.65	8.43	8.79	**8.55**
	Sodium alginate		8.64	8.44	8.8	**8.45**
	K-carrageenan		8.39	8.38	8.45	**8.05**
Bifidobacterium breve ISO8	Non-capsulated	3	8.76	8.69	7.97	**7.53**
	Sodium alginate		8.76	8.68	7.98	**7.56**
	K-carrageenan		8.58	8.43	8.29	**7.91**
	Non-capsulated	5	8.77	8.69	7.89	**7.47**
	Sodium alginate		8.46	8.68	7.93	**7.43**
	K-carrageenan		8.59	8.45	7.94	**7.76**
	Non-capsulated	6	8.77	8.67	8.54	**8.78**
	Sodium alginate		8.76	8.67	8.57	**8.77**
	K-carrageenan		8.58	8.45	8.24	**7.99**
	Non-capsulated	7	8.76	8.77	8.78	**8.77**
	Sodium alginate		8.77	8.76	8.78	**8.79**
	K-carrageenan		8.58	8.69	8.69	**8.7**
	Non-capsulated	8	8.76	8.75	8.37	**7.53**
	Sodium alginate		8.76	8.74	8.39	**6.9**
	K-carrageenan		8.58	8.64	8.54	**8.37**
Leuconostoc mesenteroides subsp. *mesenteroides* DSM 20343	Non-capsulated	3	7.98	7.84	7.86	**7.87**
	Sodium alginate		7.99	7.83	7.85	**7.87**
	K-carrageenan		7.85	7.62	7.32	**7.30**
	Non-capsulated	5	7.89	7.80	7.82	**7.87**
	Sodium alginate		7.87	7.81	7.82	**7.87**
	K-carrageenan		7.96	7.63	7.31	**7.29**
	Non-capsulated	6	7.85	7.86	7.87	**7.89**
	Sodium alginate		7.85	7.86	7.86	**7.89**
	K-carrageenan		7.83	7.62	7.30	**7.28**
	Non-capsulated	7	8.28	8.34	8.35	**8.38**
	Sodium alginate		8.27	8.35	8.36	**8.39**
	K-carrageenan		8.10	8.28	8.33	**8.29**
	Non-capsulated	8	8.26	8.36	8.37	**8.89**
	Sodium alginate		8.26	8.36	8.37	**8.40**

	K-carrageenan	8.23	8.27	8.30	**8.28**

The findings align with those of **Andrade *et al.* (2019)**, who reported that microencapsulated *Lb. brevis* CCMA1284 and *Lb. plantarum* CCMA0359 cells, utilizing various matrix combinations, exhibited greater protection compared to their free cell counterparts. Additionally, certain research indicates that the tolerance of probiotic bacteria to reduced pH levels is strain-specific (**Ramos *et al.*, 2013**), which supports the outcomes of the current investigation.

Phenol tolerance:

The survival rates of free and encapsulated LAB, prepared under optimized conditions, in phenol over a 6-hour exposure period are presented in **Table 3**. The findings from this study indicated that the viability of both free and encapsulated cells diminished as the phenol concentration increased from 0.1% to 0.5% after 6 hours. Notably, the survival of all bacterial tested strains remained above 10^7 CFU ml^{-1}, throughout the 6-hour incubation at 37 °C. The data revealed that the survival of free cells decreased by one Log CFU ml^{-1} at both 0.1% and 0.5% phenol concentrations within the first 2 hours. At phenol concentrations of 0.1% and 0.3%, there was a minor reduction in the counts of encapsulated LAB after 4 hours of incubation, in comparison to free cells under the same conditions. Conversely, the survival of encapsulated LAB was adversely affected as the phenol concentration increased from 0.3% to 0.5% after 6 hours. Specifically, at 0.3% and 0.5% phenol, there was a 2-log reduction in the counts of free LAB cells following 6 hours of incubation. Survival rates in phenol at 0.3% were superior to those at 0.5% for both strains. Nevertheless, all strains maintained a count above the acceptable threshold of 10^6 CFU ml^{-1}.

The results obtained are partially consistent with the findings of **Abd El-Salam *et al.* (2004)**, who reported that the strains *Lb. acidophilus* TISTR450, *Lb. johnsonii* ATCC33200, and *Lb. acidophilus* ATCC20552 demonstrated significant tolerance to phenol. Additionally, **Acharya and Shah (2002)** observed that isolates of LAB were capable of growth in the presence of 0.2% phenol. A study was conducted to assess the impact of phenol, *p*-cresol, and indole (at doses of 2, 20, and 100 µg/ml) on the growth and survival of four strains of intestinal LAB. The results indicated that the tested concentrations of phenol and *p*-cresol did not affect bacterial growth. However, the growth of two strains experienced slight inhibition when exposed to 100 µg/ml of indole. The survival of LAB remained unaffected by phenol and *p*-cresol for up to 120 hours, although a strain-dependent response to carcinogens was observed after this incubation period. Indole concentrations of 20 and 100 µg/ml were found to be toxic to all tested strains, but this toxicity was evident only after 24, 48, or 72 hours of incubation, depending on the specific strain. In contrast, a concentration of 2 µg/ml of indole had minimal impact (**Nowak and Libudzisz, 2006**). Furthermore, **Iqbal *et al.* (2021)** identified Lactobacillus strains *as Lactobacillus fermentum* MGA23-1 and *Lactobacillus fermentum* LMEM19, which exhibited resistance to inhibitory substances such as phenol at a concentration of 0.2%.

Table 3. Survival (Log CFU ml^{-1}) of non-encapsulated and encapsulated lactic acid bacteria up to 6 hours of exposure to different phenol concentrations at 37°C in MRS medium.

Strains	Materials	Phenol (%)	Incubation time (hours)			
			Zero	2	4	6
Lb. plantarum ATCC14917	Non-capsulated	Zero	8.66	8.65	8.74	**8.85**
	Sodium alginate		8.69	8.63	8.74	**8.83**
	K-carrageenan		8.49	8.6	8.75	**8.79**
	Non-capsulated	0.1	8.66	8.33	7.86	**7.69**
	Sodium alginate		8.67	8.36	7.85	**7.62**
	K-carrageenan		8.5	8.45	7.89	**7.69**
	Non-capsulated	0.2	8.66	8.34	7.77	**7.47**
	Sodium alginate		8.66	8.41	7.8	**7.46**
	K-carrageenan		8.49	8.42	7.84	**7.53**
	Non-capsulated	0.3	8.65	8.17	7.84	**7.04**
	Sodium alginate		8.66	8.19	7.83	**6.95**
	K-carrageenan		8.5	8.2	7.82	**6.77**
	Non-capsulated	0.4	8.49	8.14	7.6	**7.27**
	Sodium alginate		8.52	8.12	7.69	**7.11**
	K-carrageenan		8.5	8.11	7.69	**7.04**
	Non-capsulated	0.5	8.39	8.09	7.47	**6.7**
	Sodium alginate		8.36	7.99	7.6	**6.6**
	K-carrageenan		8.5	7.98	7.61	**6.74**

Lb. casei NCAIM B01137	Non-capsulated	Zero	8.66	8.83	8.87	**8.9**
	Sodium alginate		8.67	8.83	8.85	**8.89**
	K-carrageenan		8.50	8.43	8.36	**8.73**
	Non-capsulated	0.1	8.38	7.60	7.49	**7**
	Sodium alginate		7.65	7.30	6.94	**6.95**
	K-carrageenan		7.53	7.47	7.14	**7**
	Non-capsulated	0.2	8.19	7.59	7.51	**7.00**
	Sodium alginate		7.70	7.30	7.04	**6.69**
	K-carrageenan		7.55	7.46	7.11	**6.95**
	Non-capsulated	0.3	8.35	7.47	7.00	**6.77**
	Sodium alginate		8.21	7.30	6.77	**6.6**
	K-carrageenan		7.44	7.38	6.47	**6.30**
	Non-capsulated	0.4	8.00	7.39	6.69	**6.54**
	Sodium alginate		7.62	6.95	6.58	**6.49**
	K-carrageenan		7.46	7.27	6.78	**6.30**
	Non-capsulated	0.5	7.84	6.84	6.32	**6.00**
	Sodium alginate		6.94	6.79	6	**5.98**
	K-carrageenan		7.30	6.75	6.29	**5.01**
Lb. rhamnosus ISO7	Non-capsulated	Zero	8.58	8.83	8.91	**8.99**
	Sodium alginate		8.59	8.81	8.9	**8.98**
	K-carrageenan		8.49	8.64	8.69	**8.75**
	Non-capsulated	0.1	8.55	8.45	8.09	**7.96**
	Sodium alginate		8.57	8.43	8.07	**7.97**
	K-carrageenan		8.48	8.38	7.99	**7.67**
	Non-capsulated	0.2	8.54	8.43	8.02	**7.86**
	Sodium alginate		8.53	8.42	8.01	**7.8**
	K-carrageenan		8.48	8.36	7.75	**7.38**
	Non-capsulated	0.3	8.56	8.35	7.99	**7.87**
	Sodium alginate		8.58	8.34	7.97	**7.80**
	K-carrageenan		8.39	8.17	7.62	**7.30**
	Non-capsulated	0.4	8.43	8.22	7.94	**7.75**
	Sodium alginate		8.5	8.21	7.89	**7.71**
	K-carrageenan		8.33	8.18	7.5	**7.07**
	Non-capsulated	0.5	8.49	8.21	7.93	**7.63**
	Sodium alginate		8.48	8.2	7.93	**7.62**
	K-carrageenan		8.29	8.08	7.43	**7.04**
Bifidobacterium breve ISO8	Non-capsulated	Zero	8.49	8.73	8.82	**8.85**
	Sodium alginate		8.51	8.74	8.83	**8.85**
	K-carrageenan		8.34	8.56	8.68	**8.7**
	Non-capsulated	0.1	8.46	8.38	7.75	**7.46**
	Sodium alginate		8.5	8.36	7.77	**7.53**
	K-carrageenan		8.32	8.13	7.59	**7.27**
	Non-capsulated	0.2	8.45	8.38	7.71	**7.54**
	Sodium alginate		8.49	8.36	7.67	**7.43**
	K-carrageenan		8.32	8.12	7.46	**7.07**
	Non-capsulated	0.3	8.47	8.35	7.65	**7.32**
	Sodium alginate		8.47	8.33	7.63	**7.27**
	K-carrageenan		8.29	8.06	7.49	**6.95**
	Non-capsulated	0.4	8.46	8.32	7.67	**7.04**
	Sodium alginate		8.49	8.32	7.71	**7.46**
	K-carrageenan		8.04	7.97	7.27	**6.90**
	Non-capsulated	0.5	8.45	8.32	7.62	**7.34**
	Sodium alginate		8.45	8.33	7.62	**7.43**
	K-carrageenan		7.94	7.65	7.04	**6.3**
Leuconostoc mesenteroides subsp. *mesenteroides* DSM 20343	Non-capsulated	Zero	8.29	8.38	8.89	**8.93**
	Sodium alginate		8.29	8.37	8.90	**8.95**
	K-carrageenan		8.01	8.38	8.24	**8.89**
	Non-capsulated	0.1	8.03	7.86	7.60	**7.42**
	Sodium alginate		8.03	7.86	7.59	**7.42**
	K-carrageenan		7.99	7.77	7.62	**7.57**

	Non-capsulated	0.2	8.04	7.80	7.42	**7.32**
	Sodium alginate		8.04	7.81	7.40	**7.31**
	K-carrageenan		7.99	7.70	7.61	**7.21**
	Non-capsulated	0.3	8.04	7.79	7.28	**7.20**
	Sodium alginate		8.04	7.79	7.28	**7.19**
	K-carrageenan		7.98	7.71	7.26	**7.19**
	Non-capsulated	0.4	8.04	7.79	7.16	**7.02**
	Sodium alginate		8.04	7.78	7.16	**7.06**
	K-carrageenan		7.98	7.70	7.01	**6.00**
	Non-capsulated	0.5	8.03	7.70	7.09	**6.99**
	Sodium alginate		8.03	7.71	7.09	**6.98**
	K-carrageenan		7.98	7.70	6.97	**5.75**

Antibiotic susceptibility:

Table 4 illustrates the susceptibility profiles of all LAB isolates in relation to commonly utilized antibiotics. The antibiotic resistance of LAB was assessed through disk diffusion methods. The susceptibility of antibiotics is regarded as a vital safety consideration for probiotic strains that lack antibiotic resistance. Antibiotics exert their effects through two distinct mechanisms of action. ***Firstly***, cell wall inhibitors *i.e.*, Ampicillin and Cefotaxime. In the present study (**Table 4**), all LAB strains were sensitive to Ampicillin, but all LAB strains were moderately susceptible to Cefotaxime, except *Lb. plantarum* was sensitive to Cefotaxime. ***Secondly***, the protein synthesis inhibitors *i.e.*, Gentamycin, Kanamycin, Erythromycin and Lincomycin. **Table 4** shows that 100% of tested LAB strains showed resistance to Gentamycin, Kanamycin, Erythromycin and Lincomycin.

Concerns regarding the safety of probiotic products include the issue of antibiotic resistance (**Yeo *et al*., 2015**). Additionally, it is recognized that probiotics possess genetic traits that facilitate the emergence of various forms of antibiotic resistance (**Zhang *et al*., 2017**). The findings presented in **Table 4** who indicated that the tested LAB strains exhibited varying levels of resistance to many of the antibiotics assessed. Notably, all strains demonstrated complete resistance to Ampicillin, Gentamycin, Kanamycin, Erythromycin, and Lincomycin. **Naeem *et al*. (2012)** who conducted an antibiotic susceptibility analysis on 15 isolates against 10 antibiotics, revealing that all isolates were sensitive to at least 50% of the antibiotics tested, with the highest sensitivity recorded for Oxacillin and Kanamycin. These results are consistent with the findings of **Väkeväinen *et al*. (2018); Sirichoat *et al*. (2020)** and **Xu *et al*. (2020).**

Table 4. Antibiotic resistance profiles of some lactic acid bacteria strains

Tested strains	Diameter of inhibition zone (mm)					
	K	**L**	**G**	**C**	**A**	**E**
***Lb. casei* NCAIM B01137**	35	54	47	18	44	52
***Lb. plantarum* ATCC14917**	35	55	45	24	40	55
***Bifidobacterium breve* ISO8**	40	62	48	20	31	65
***Lb. rhamnosus* ISO7**	33	63	60	22	37	62
***Leuconostoc mesenteroides* subsp. *mesenteroides* DSM 20343**	39	64	54	15	30	75

K: Kanamycin; **L**: Lincomycin; **G**: Gentamycin; **C**: Cefotaxime; **A**: Ampicillin; **E**: Erythromycin

Salt (NaCl) tolerance:

Table (5) illustrates the viability of free and encapsulated LAB cells in their tolerance to various concentrations of NaCl after 6 hours. The viability of all tested LAB strains ranged from 7.98 to 8.76 Log CFU g^{-1} in zero time of incubation. Results cleared that a little decline in the viability of all the tested LAB strains occurred as the concentration of NaCl increased. The survival of LAB strains varied from 7.30 to 8.86 Log CFU g^{-1} after 6 hours of incubation time. The overall viability of all LAB strains diminished as the salt concentration increased during the incubation period of up to 6 hours. This decline in survival can be attributed to a decrease in water activity and an increase in osmolarity resulting from the elevated salt concentration **(Atallah, 2013)**. At 4% NaCl, changes in the survival of non-encapsulated and encapsulated LAB cells were pronounced after 6 hours of incubation time.

Table 5. Viability (Log CFU ml $^{-1}$) of non-encapsulated and encapsulated LAB up to 6 hours of exposure to different NaCl concentrations at 37°C in MRS medium.

Strains	Materials	NaCl (%)	Incubation time (hours)			
			Zero	2	4	6
Lb. plantarum ATCC14917	Non-capsulated	Zero	8.73	8.76	8.77	**8.80**
	Sodium alginate		8.74	8.76	8.77	**8.80**
	K-carrageenan		8.62	8.68	8.59	**8.77**
	Non-capsulated	1	8.73	8.74	8.64	**8.63**
	Sodium alginate		8.74	8.68	8.65	**8.62**
	K-carrageenan		8.63	8.61	8.58	**8.51**
	Non-capsulated	2	8.73	8.68	8.64	**8.66**
	Sodium alginate		8.73	8.69	8.61	**8.62**
	K-carrageenan		8.63	8.61	8.57	**8.49**
	Non-capsulated	4	8.74	8.68	8.64	**8.60**
	Sodium alginate		8.74	8.68	8.62	**8.59**
	K-carrageenan		8.62	8.59	8.55	**8.47**
Lb. casei NCAIM B01137	Non-capsulated	Zero	8.71	8.78	8.83	**8.82**
	Sodium alginate		8.70	8.8	8.78	**8.78**
	K-carrageenan		8.51	8.67	8.71	**8.73**
	Non-capsulated	1	8.67	8.32	8.18	**7.73**
	Sodium alginate		8.60	8.30	8.05	**7.97**
	K-carrageenan		8.49	8.16	7.98	**7.91**
	Non-capsulated	2	8.66	8.31	8.00	**7.92**
	Sodium alginate		8.59	8.46	7.97	**7.69**
	K-carrageenan		8.47	8.16	7.85	**7.69**
	Non-capsulated	4	8.57	8.24	8.00	**7.67**
	Sodium alginate		8.54	8.19	7.76	**7.63**
	K-carrageenan		8.44	8.11	7.85	**7.55**
Lb. rhamnosus ISO7	Non-capsulated	Zero	8.72	8.77	8.78	**8.83**
	Sodium alginate		8.72	8.78	8.78	**8.86**
	K-carrageenan		8.62	8.58	8.54	**8.49**
	Non-capsulated	1	8.73	8.67	8.65	**8.64**
	Sodium alginate		8.73	8.67	8.64	**8.63**
	K-carrageenan		8.61	8.57	8.54	**8.49**
	Non-capsulated	2	8.73	8.66	8.64	**8.65**
	Sodium alginate		8.72	8.63	8.62	**8.63**
	K-carrageenan		8.61	8.56	8.51	**8.44**
	Non-capsulated	4	8.73	8.65	8.63	**8.59**
	Sodium alginate		8.72	8.62	8.61	**8.61**
	K-carrageenan		8.61	8.56	8.5	**8.39**
Bifidobacterium breve ISO8	Non-capsulated	Zero	8.76	8.77	8.78	**8.79**
	Sodium alginate		8.75	8.77	8.77	**8.80**
	K-carrageenan		8.65	8.69	8.69	**8.73**
	Non-capsulated	1	8.72	8.60	8.53	**8.27**
	Sodium alginate		8.73	8.62	8.54	**8.13**
	K-carrageenan		8.65	8.51	8.39	**8.11**
	Non-capsulated	2	8.70	8.54	8.17	**7.88**
	Sodium alginate		8.71	8.49	7.93	**7.60**
	K-carrageenan		8.61	8.51	8.27	**7.96**
	Non-capsulated	4	8.69	8.45	8.04	**7.61**
	Sodium alginate		8.68	8.45	7.86	**7.38**
	K-carrageenan		8.67	8.45	8.87	**7.61**
Leuconostoc mesenteroides subsp. *mesenteroides* DSM 20343	Non-capsulated	Zero	8.27	8.34	8.33	**8.43**
	Sodium alginate		8.26	8.38	8.35	**8.47**
	K-carrageenan		8.21	8.22	8.20	**8.31**
	Non-capsulated	1	8.24	8.17	8.11	**7.81**
	Sodium alginate		8.26	8.20	8.08	**7.89**
	K-carrageenan		7.98	7.90	7.82	**7.62**
	Non-capsulated	2	8.24	8.15	8.07	**7.68**

	Sodium alginate		8.25	8.19	8.07	**7.70**
	K-carrageenan		7.98	7.89	7.71	**7.42**
	Non-capsulated	4	8.23	8.13	7.99	**7.46**
	Sodium alginate		8.25	8.17	7.97	**7.60**
	K-carrageenan		7.98	7.80	7.50	**7.30**

Also, the results in this study showed that *Leuconostoc mesenteroides* subsp. *mesenteroides* DSM 20343 was more affected by NaCl concentrations during incubation time. These results may suggest that *Lb. plantarum* ATCC14917, *Lb. rhamnosus* ISO7 and *Bifidobacterium breve* ISO8 strains have a greater physiological capacity to tolerant high concentration of NaCl than *Lb. casei* NCAIM B01137. These results agree with those of **Vinderola *et al.* (2002)**. In addition, most of LAB used as starter is sensitive to NaCl concentrations higher than 2.5%. Salt is considered a limiting factor for viability and growth of probiotics in most of white brined cheese **(Özer *et al.*, 2009).** The addition of ahigh quantity of NaCl (5.4 to 9.5% NaCl) in manufacturing of Domiati cheese is considered a major factor in using it as vehicle for the delivery of probiotic bacteria. **Hajmeer *et al.* (2006)** reported that salt reduces or inhibits microbial growth by changing in water activity, ionic strength and exerting a drying effect on microorganisms. The data obtained was in harmony with those of **Sabikhi *et al.* (2010)** for their studied on different strains of probiotics. The findings indicated that the encapsulation of LAB strains with sodium alginate and k-carrageenan could potentially improve their resistance to NaCl.

Conclusion

All LAB strains exhibited robust growth in the presence of 0.1% and 0.5% bile salt during incubation periods up to 6 hours, except for *Leuconostoc mesenteroides* subsp. *mesenteroides* DSM 20343. At a pH of 7.0, the viability of LAB in MRS medium remained stable after 6 hours of incubation time at 37°C, regardless of whether the cells were encapsulated or non- encapsulated. Conversely, at pH 3, there was a minor reduction in the number of encapsulated LAB cells after 6 hours, while free cells experienced a more significant decline by one log cycle under the same conditions. The survival rate of all LAB strains diminished as the NaCl concentration increased during the 6-hour incubation period. The LAB strains demonstrated resistance to Gentamycin, Kanamycin, Erythromycin, and Lincomycin. Additionally, it was observed that capsules made from sodium alginate and k-carrageenan offered protection to the tested strains against gastric acid, resulting in a higher release of viable bacteria in the intestine.

References

Abd El-Salam, B.A.; Sultan, N.I.; Fayed, E.O. and Zedan, M.A. (2004). *In vitro* studies on probiotic criteria of some lactobacilli. *Egypt. J. Dairy Sci.*, 32:17-29.

Abd El-Salam, M.H. and El-Shibiny, S. (2015). Preparation and properties of milk proteins-based encapsulated probiotics: a review. *Dairy science and technology*, 95: 393-412.

Abedfar, A.; Hosseininezhad, M.; Sadeghi, A. and Raeisi, M. (2021). Comparative survival of exopolysaccharide encapsulated *Lactobacillus plantarum* and *Pediococcus pentosaceus* in simulated gastrointenstinal conditions and storage time. Journal of Food Measurement and Characterization, 15: 594-603.

Acharya, M.R. and Shah, R.K. (2002). Selection of human isolates of Bifidobacteria for their use as probiotics. *Applied biochemistry and biotechnology*, *102*: 81-98.

Ali, U.; Saeed, M.; Ahmad, Z.; Shah, F.U.H.; Rehman, M.A.; Mehmood, T. and Rahman, A. (2023). Stability and survivability of alginate gum-coated *Lactobacillus rhamnosus* GG in simulated gastrointestinal conditions and probiotic juice development. *Journal of Food Quality*, 2023(1): 3660968.

Andrade, D.P.; Ramos, C.L.; Botrel, D.A.; Borges, S.V.; Schwan, R.F. and Ribeiro Dias, D. (2019). Stability of microencapsulated lactic acid bacteria under acidic and bile juice conditions. *International Journal of Food Science & Technology*, 54(7): 2355-2362.

Atallah, A.A. (2013). Technological Aspects on Functional Dairy Starter Cultures. Ph.D. Thesis, Benha Univ. Egypt.

Azam, M.; Saeed, M.; Yasmin, I.; Afzaal, M.; Ahmed, S.; Khan, W.A. and Asif, M. (2021). Microencapsulation and invitro characterization of *Bifidobacterium animalis* for improved survival. *Journal of Food Measurement and Characterization*, 15: 2591-2600.

Basyigit, G.; Kuleasan, H. and Karahan, A.G. (2006). Viability of human-derived probiotic lactobacilli in ice cream produced with sucrose and aspartame. *J. Ind. Microbial. Biotechnol.*, 33:796-800.

Burgain, J.; Gaiani, C.; Linder, M. and Scher, J. (2011). Encapsulation of probiotic living cells: From laboratory scale to industrial applications. *Journal of food engineering*, 104(4): 467-483.

Chandramouli, V.; Kailasapathy, K.; Peiris, P. and Jones, M. (2004). An improved method of microencapsulation and its evaluation to protect *Lactobacillus* spp. in simulated gastric conditions. *Journal of microbiological methods*, 56(1): 27-35.

Dumitru, M.; Lefter, N.A.; Habeanu, M.; Ciurescu, G.; Vodnar, D.C.; Elemer, S.; and Dudu, A. (2023). Evaluation of lactic acid bacteria isolated from piglets tract and encapsulation of selected probiotic cells. *Agriculture*, 13(5): 1098.

Hajmeer, M.; Ceylan, E.; Marsden, J.L. and Fung, D.Y. (2006). Impact of sodium chloride on *Escherichia coli* O157: H7 and *Staphylococcus aureus* analysed using transmission electron microscopy. *Food Microbiology*, 23(5): 446-452.

Iqbal, A.; Irshad, S.; Saeed, S. and Tareen, A. (2021). Probiotic potential of encapsulated *Lactobacillus* species in yogurt formation indigenously isolated from dairy source. *Advancements in Life Sciences*, 8(3): 267-274.

Kailasapathy, K. (2002). Microencapsulation of probiotic bacteria: technology and potential applications. *Current issues in intestinal microbiology*, 3(2): 39-48.

Kebary, K.M.K.; Hussein, S.A. and Badawi, R.M. (1998). Improving viability of bifidobacteria and their effect on frozen ice milk. *Egyptian Journal of Dairy Science*, 26(2): 319-337.

Kociubinski, G.; Pérez, P.; and De Antoni, G. (1999). Screening of bile resistance and bile precipitation in lactic acid bacteria and bifidobacteria. *Journal of food protection*, 62(8): 905-912.

Lin, M.Y. and Chen T.W. (2000). Reduction of cholesterol by *lactobacillus acidophilus* in culture broth. *J. Food and Drag Analysis*, 8:97-102.

Mahmoud, M.; Abdallah, N.A.; El-Shafei, K.; Tawfik, N.F. and El-Sayed, H.S. (2020). Survivability of alginate-microencapsulated *Lactobacillus plantarum* during storage, simulated food processing and gastrointestinal conditions. *Heliyon*, 6 (2020): e03541. https://doi.org/10.1016/j.heliyon.2020.e03541.

Mahmoud, S.Y.; Atallah, A.A.; Badr, O.A.; Moustafa, M.M.; Esmael, A.; Ebrahim, N. and Mohamed, S.A. (2022). Bioprospecting for novel probiotic strains from human milk and infants: Molecular, Biochemical, and Ultrastructural Evidence. *Biology*, 11(10): 1405.

Mu, J.; Qu, L.; Jin, F.; Zhang, S.; Fang, C.; Ma, X. and Wang, J. (2018). Abundance and distribution of micro plastics in the surface sediments from the northern Bering and Chukchi Seas. *Environmental Pollution*, 245: 122-130.

Naeem, M.; Ilyas, M.; Haider, S.; Baig, S. and Saleem, M. (2012). Isolation characterization and identification of lactic acid bacteria from fruit juices and their efficacy against antibiotics. *Pak. J. Bot*, 44(8).

Nowak, A., and Libudzisz, Z. (2006). Influence of phenol, p-cresol and indole on growth and survival of intestinal lactic acid bacteria. *Anaerobe*, 12(2): 80-84.

Özer, B.; Kirmaci, H.A. Şenel, E.; Atamer, M. and Hayaloğlu, A. (2009). Improving the viability of *Bifidobacterium bifidum* BB-12 and *Lactobacillus acidophilus* LA-5 in white-brined cheese by microencapsulation. *International Dairy Journal*, 19(1): 22-29.

Pradipta, I.; Kong, D. and Tan, J.B.L. (2019). Natural organic antioxidants from green tea inhibit corrosion of steel reinforcing bars embedded in mortar. *Construction and Building Materials*, 227: 117058.

Prevost, H. and Divies, C. (1992). Cream fermentation by a mixed culture of lactococci entrapped in two-layer calcium alginate gel beads. *Biotechnology letters*, 14: 583-588.

Pupa, P.; Apiwatsiri, P.; Sirichokchatchawan, W.; Pirarat, N.; Muangsin, N.; Shah, A.A. and Prapasarakul, N. (2021). The efficacy of three double-microencapsulation methods for preservation of probiotic bacteria. *Scientific reports*, 11(1): 13753.

Ramos, C.L.; Thorsen, L.; Schwan, R.F. and Jespersen, L. (2013). Strain-specific probiotics properties of *Lactobacillus fermentum*, *Lactobacillus plantarum* and *Lactobacillus brevis* isolates from Brazilian food products. *Food microbiology*, 36(1): 22-29.

Sabikhi, L.; Babu, R.; Thompkinson, D.K. and Kapila, S. (2010). Resistance of microencapsulated *Lactobacillus acidophilus* LA1 to processing treatments and simulated gut conditions. *Food and Bioprocess Technology*, 3: 586-593.

Shah, N.P and Lankaputhra, W.E.V. (2002). Bifidobacterium spp. (Morphology and Physiology Applications in Fermented Milks). *Encyclopedia of Dairy Sciences*, 141-146.

Sheu, T.Y. and Marshall, R.T. (1993). Micro entrapment of lactobacilli in calcium alginate gels. *Journal of food science*, 58(3): 557-561.

Shi, L.E.; Zheng, W.; Zhang, Y. and Tang, Z.X. (2016). Milk-alginate microspheres: Protection and delivery of *Enterococcus faecalis* HZNU

P2. *LWT-Food Science and Technology*, 65: 840-844.

Sirichoat, A.; Flórez, A.B.; Vázquez, L.; Buppasiri, P.; Panya, M.; Lulitanond, V. and Mayo, B. (2020). Antibiotic susceptibility profiles of lactic acid bacteria from the human vagina and genetic basis of acquired resistances. *International Journal of Molecular Sciences*, 21(7): 2594.

Soukoulis, C.; Cappellin, L.; Aprea, E.; Costa, F.; Viola, R.; Märk, T.D. and Biasioli, F. (2013). PTR-ToF-MS, a novel, rapid, high sensitivity and non-invasive tool to monitor volatile compound release during fruit post-harvest storage: the case study of apple ripening. *Food and Bioprocess Technology*, 6: 2831-2843.

Suskovic, J.; Brkić, B.; Matošić, S. and Maric, V. (1997). *Lactobacillus acidophilus* M92 as potential probiotic strain. *Milchwissenschaft*, 52:430-435.

Väkeväinen, K.; Valderrama, A.; Espinosa, J.; Centurión, D.; Rizo, J.; Reyes-Duarte, D. and Plumed-Ferrer, C. (2018). Characterization of lactic acid bacteria recovered from atole agrio, a traditional Mexican fermented beverage. *Lwt*, 88: 109-118.

Vinderola, C.G.; Mocchiutti, P. and Reinheimer, J.A. (2002). Interactions among lactic acid starter and probiotic bacteria used for fermented dairy products. *Journal of Dairy science*, 85(4): 721-729.

Xu, Y.; Zhou, T.; Tang, H.; Li, X.; Chen, Y.; Zhang, L. and Zhang, J. (2020). Probiotic potential and amylolytic properties of lactic acid bacteria isolated from Chinese fermented cereal foods. *Food Control*, 111: 107057.

Yeo, S.; Lee, S.; Park, H.; Shin, H.; Holzapfel, W. and Huh, C.S. (2015). Development of putative probiotics as feed additives: validation in a porcine-specific gastrointestinal tract model. *Applied microbiology and biotechnology*, 100(23): 10043-10054.

Zhang, J.; Xiao, X.; Dong, Y.; Shi, L.; Xu, T.; and Wu, F. (2017). The anti-obesity effect of fermented barley extracts with *Lactobacillus plantarum* dy-1 and *Saccharomyces cerevisiae* in diet-induced obese rats. *Food Funct*. 8: 1132–1143. https://doi.org/ 10.1039/C6FO01350C.