Agriculture and Food

AIMS Agriculture and Food, 9(2): 483–499.
DOI: 10.3934/agrfood.2024028
Received: 15 February 2024
Revised: 25 March 2024
Accepted: 25 March 2024
Published: 24 April 2024

https://www.aimspress.com/journal/agriculture

Research article

Comparison of probiotic properties between free cells and encapsulated cells of *Limosilactobacillus fermentum* InaCC B1295

Usman Pato[1,*], Yusmarini Yusuf[1], Emma Riftyan[1], Evy Rossi[1], and Agrina[2]

[1] Faculty of Agriculture, Universitas Riau, Pekanbaru, Riau 28293, Indonesia
[2] Faculty of Nursing, Universitas Riau, Pekanbaru, Riau 28293, Indonesia

***Correspondence:** Email: usmanpato@yahoo.com; usman.pato@lecturer.unri.ac.id; Tel: +628127639712.

Abstract: Probiotics are microflora that can improve intestinal health and the immune system, positively impacting human health. This study aimed to evaluate the ability of free cells and *Limosilactobacillus fermentum* InaCC B1295 (LFB1295) cells encapsulated with cellulose microfiber hydrogel (CMFH) from oil palm fronds (OPF) against gastric acid, bile ox gall, autoaggregation, coaggregation, and hydrophobicity of surface cells to reach the columns with high viability numbers and be capable of attaching to and colonizing the colon. The research was carried out experimentally by referring to previous research methods. Research data in resistance to gastric acid and bile salts, autoaggregation, coaggregation, and cell surface hydrophobicity were analyzed statistically using the t-test and displayed in table and figure form. The results showed that free cells were more susceptible to gastric acid and bile salts than CMFH-encapsulated cells from OPF, as indicated by a much more promising reduction in the viability of free cells compared to CMFH-encapsulated LFB1295 cells from OPF. Hence, LFB1295 free cells had higher autoaggregation, cell surface hydrophobicity, and coaggregation values than CMGH-encapsulated cells from OPF. Free and encapsulated cells generally have high coaggregation values with fellow lactic acid bacteria (LAB), *Pediococcus pentosaceus*, compared to coaggregation with pathogenic bacteria, namely *S. aureus* and *E. coli*. These findings indicate that free cells or cells encapsulated with CMFH-OPF have excellent acid and bile salts, autoaggregation, coaggregation, and hydrophobicity and qualify as probiotics.

Keywords: probiotic properties; cellulose microfiber; *Limosilactobacillus fermentum*; *Pediococcus pentosaceus*; encapsulation

1. Introduction

Probiotics are living microorganisms that, when ingested in adequate amounts, can benefit the health of their hosts. Probiotics will improve the balance of intestinal microflora and inhibit the growth of pathogens in the digestive tract, thereby reducing and preventing various diseases. Microorganisms classified as probiotics are generally recognized as safe (GRAS), namely microorganisms with a very low possibility of infection and ones that do not produce toxins [1]. The genera of microorganisms commonly used as probiotic agents are Lactobacillus, Pediococcus, Lactococcus, Leuconostoc, and Bifidobacterium [2,3].

The bacterial cells can be encapsulated to preserve the probiotic's vitality. Encapsulating cells involves covering them with substances that shield them from harsh surroundings. These days, probiotic particles are created using a variety of encapsulating processes. It is imperative to consider the need for a non-aggressive process when selecting a technique, as this will guarantee enough viability of the encapsulated cells and mechanical stability appropriate for the intended use. Nowadays, probiotic cells are encapsulated using a variety of methods, including coacervation, electrospraying, extrusion, emulsion, spray drying, spray chilling, fluidized bed, freeze drying, and spray freeze drying [4–8]. The type of encapsulant material used will influence the encapsulation results. Encapsulants must be able to protect cells, be safe for consumption, and have an economical price. Encapsulant materials can be obtained from various natural polymers, such as carbohydrates in starch, chitosan, alginate, sucrose, pectin, and cellulose. Cellulose is a natural polymer and is an essential structural component of plant cell walls [9]. One of the materials used to make encapsulants is cellulose in cellulose microfiber (CMF). As an encapsulant material, cellulose microfiber has physical characteristics such as high strength and stiffness, and it is lightweight and biodegradable [10]. Cellulose microfiber is generally made from cellulose and is found in various parts of oil palm plants, such as the fronds.

Indonesia is the largest palm oil-producing country in the world, with a planting area for palm oil in 2023 that was 16,833,985 hectares, with a total production of 48,235,405 tons. Large plantation areas produce oil palm biomass waste, primarily stems resulting from replanting. Oil palm fronds (OPF) are a form of biomass waste that contain much cellulose [11]. According to Pato et al. [12], OPF has a composition of 67.0% cellulose, 25.4% hemicellulose, and 6.7% lignin. OPF cellulose has physical characteristics that are resistant to heat and acid, has a high surface area, and is light, so it can be processed into CMF, which is used as an encapsulant material for probiotics. Some probiotics that can be encapsulated using CMF from oil palm stems are *L. fermentum* InaCC B1295 (LFB1295). *Limosilactobacillus fermentum* is a well-studied strain with documented probiotic properties. *L. fermentum* LFB1295 was isolated from dadih, fermented buffalo milk from the Indonesian regions of Riau and West Sumatra [13]. It is acknowledged that *Limosilactobacillus fermentum* has considerable potential as a probiotic, exhibiting probiotic and antiviral activity, bio-preservative properties, and immunobiosis, as well as being classified as a safe microorganism [14–17]. Encapsulated cells will increase the viability of LFB1295. LFB1295 encapsulated CMF from oil palm trunks showed a 0.78 log CFU/g reduction in viability with low acid (pH-2) treatment after 35 days of storage [18]. According to research by [19], encapsulated *P. pentosaceus* Li05 showed more resistance to stomach acid conditions than non-encapsulated probiotics, with a viability decline of only 0.7–1.5 log CFU/g.

Lactic acid bacteria for use as a probiotic culture or food supplement must be acid and bile tolerant [20–22], which enables a selected strain to survive, grow, and conduct its therapeutic benefits

such as antimutagenic and anticarcinogenic activities [23,24], bile salt deconjugation and cholesterol binding properties [25–27], and immune modulation [28,29] in the intestinal tract. After passing through acidic conditions in the stomach and bile salts in the small intestine, probiotics must have the ability to adhere and compete with other bacteria, especially pathogenic bacteria in the colon [30,31].

The first step in determining LAB's capacity to adhere to the digestive system is to assess its cell surface hydrophobicity (CSH) and autoaggregation potential *in vitro* [32,33]. Panjaitan et al. [34] stated that the bacterial genus, growth medium, and surface structure affect microbial hydrophobicity. The CSH of each cell will impact the bacteria's capacity for coaggregation and autoaggregation. In order to obtain approval as a probiotic, the following aspects must be substantiated: 1) adhesion refers to the capacity of a substance to stick to mucus or human epithelial cells, 2) autoaggregation tests reveal that LAB may successfully establish and attach to the digestive tract, specifically the colon and small intestine, 3) coaggregation with pathogenic bacteria is a vital trait for preventing the dissemination of these harmful bacteria [35,36]. It is anticipated that lactic acid bacteria with probiotic properties like these can enter the small intestine and colon, attach themselves there, and begin to colonize. From there, they will carry out their therapeutic role of preventing a variety of degenerative diseases like cancer, digestive tract infections, and cardiovascular disease. Therefore, it is necessary to study bile and acid tolerance, autoaggregation, coaggregation, and surface cell hydrophobicity by probiotics. Hydrophobicity, autoaggregation, and coaggregation properties of LAB are prerequisites for probiotics. The current study aimed to assess the probiotic properties of encapsulated cells of LFB1295 with CMFH from an oil palm frond, including acid and bile tolerance, autoaggregation, coaggregation, and cell surface hydrophobicity.

2. Materials and methods

2.1. Materials and culture collection

Palm oil solid waste is oil palm fronds (OPF) obtained from PT. Multi Palma Sejahtera, Bandar Sei Kijang District, Pelalawan Regency, Riau Province. *Limosilactobacillus fermentum* InaCC B1295 was obtained from the Indonesian Culture Collection (InaCC), Research Center for Biology, Indonesian Institute of Sciences (LIPI), West Java, Indonesia. Polyvinyl alcohol (PVA) was purchased from Sigma-Aldrich, Steinheim, Germany.

The ten strains of LAB isolated from dadih used in this study were obtained from the stock culture collection of the Animal Product Microbiology, Graduate School of Agriculture, Shinshu University, Japan. All cultures were maintained by subculture in MRS broth using 1% inoculum and 18 to 20 hours of incubation at 37 ℃. Cultures were stored at 4 ℃ between transfers. Each culture was subcultured twice in MRS broth before experimental use. The flowchart of the research is presented in Figure 1.

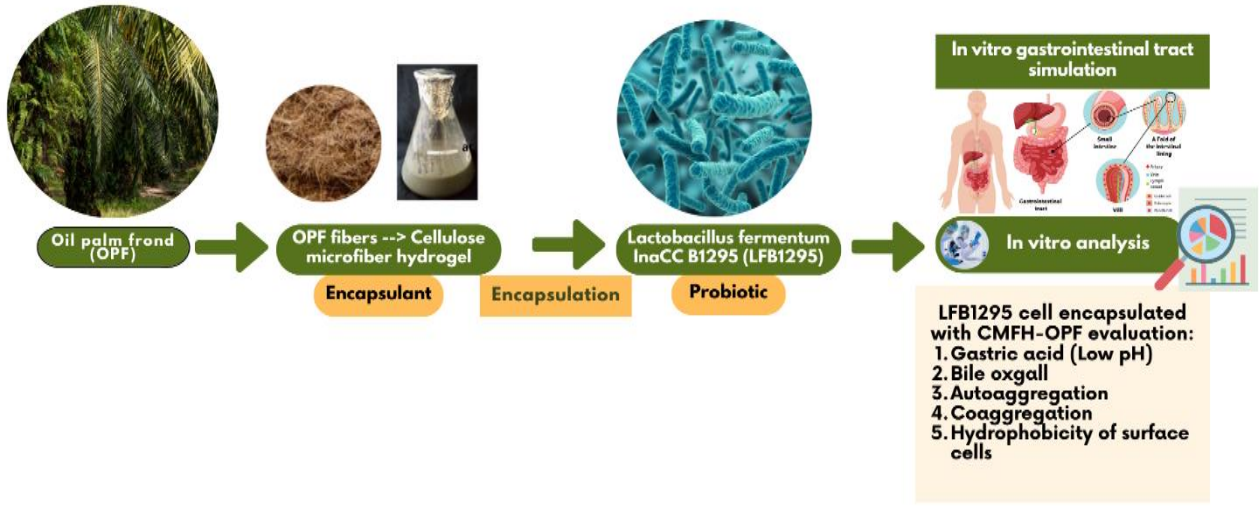

Figure 1. Flowchart of the research for LFB1295 in vitro GI tract simulation.

2.2. Preparation of the CMF from palm oil solid waste

The oil palm fronds (OPF) were fragmented, ranging in length from 0.5–1 cm, rinsed with water, and cooked for 1 hour in boiling water (100 ℃). They were then filtered. Following boiling, the OPF was thoroughly rinsed with water and dried for four hours at 60 ℃. The fiber was placed in a beaker with 1 Liter of 6% (w/v) KOH and left to soak for 12 hours at room temperature. The fibers were then washed with water three times.

Additionally, washed fibers were steeped for 5 hours in a hypochlorite solution before being filtered and rinsed with water pH 7. After drying and crushing OPF in a blender until smooth, it was filtered through a sieve with screen number 80. To avoid sample damage caused by milling heat, the CMF was handled by milling the cellulose flour at an 8000 rpm speed for 60 minutes with a run time of 15 seconds and a rest period of 2 minutes. The milled product was then sieved through a sieve with a screen number 100 to get the CMF [37].

2.3. CMF hydrogel preparation

The PVA was weighed to 96 g, then combined with 1104 mL of distilled water and heated to 100 ℃ with a hot magnetic stirrer until dissolved. The solution was allowed to be refrigerated to room temperature. Next, 250 g of PVA 8% was mixed with 250 mL of CMF palm oil frond and heated at 60 ℃ until the CMF was entirely dissolved, resulting in a CMF hydrogel (CMFH). The pH and viscosity of the CMFH were then measured using a pH meter and a viscometer. The hydrogel was autoclaved at 121 ℃ for 15 minutes. After cooling to room temperature, sterile CMFH was ready to be used as a LAB encapsulant [12].

2.4. Activation of Limosilactobacillus fermentum InaCC B1295

Lactobacillus fermentum InaCC B1295 culture was inoculated into a 5 mL MRSB medium test tube and then agitated by the vortex. The medium was incubated for 24 hours at 37 ℃ in an incubator to obtain the active culture, as indicated by measuring the absorbance (OD) of the growth medium of 1.50 at 625 nm. The active cultures were stored in the refrigerator until use [18].

2.5. Preparation of encapsulated Limosilactobacillus fermentum InaCC B1295

The encapsulation process involved adding 40 mL of cell biomass InaCC B129 containing about 4×10^{10} log CFU/mL to 40 mL of sterile CMF hydrogel from oil palm frond (CMFH-OPF) and stirring until well-blended using a stirring rod. Storage of *L. fermentum* InaCC B129 was carried out by inserting 2 mL of each of the encapsulated LAB into 5 mL cryovial, which was subsequently stored at room temperature and refrigerated temperatures (4 ℃) for 0, 14, and 28 days. Subsequently, the probiotic properties of the encapsulated *L. fermentum* InaCC B129 were assessed [12].

2.6. Assay for bile tolerance

The resistance to bile salts test of free or encapsulated cells by CMFH from OPF was carried out according to the method of [38] using de Man Rogosa and Sharpe Broth (MRSB), which rejuvenated the LAB culture for 24 h. A total culture of 0.1 mL was inoculated into 10 mL MRSB (control), and 0.3% bile salt was added, then incubated at 37 ℃ for 5 h. Afterward, the plate count method calculated the number of colonies on MRSA media. Subsequent serial dilutions were made and plated using the spread-plate method with MRS agar. The plates were incubated at 37 ℃ for 48 h before enumeration.

2.7. Assay for acid tolerance

Acid tolerance was assayed according to Baig et al. [39]. Washed cell pellets of the LAB were resuspended in sterile distilled water, and the absorbance at 625 nm was adjusted at 0.7 for each culture. Cell suspensions at the level of 2% were inoculated into each of 10 mL of 2% non-fat dry milk (NDM) that had been adjusted to pH 2.5 by 0.1 N HCl and pH 6.9 (control; without pH adjustment). The mixtures were incubated at 37 ℃ for 2 h. Immediately after the incubation period, 1 mL of suspended cells were diluted with 9 mL of 66 mM phosphate buffered saline (PBS), pH 6.8, and mixed uniformly with a vortex mixer. Subsequent serial dilutions were made and plated using the spread-plate method with MRS agar. The plates were incubated at 37 ℃ for 48 h before enumeration.

2.8. Autoaggregation activity

Autoaggregation of *L. fermentum* InaCC B1295 encapsulated with CMFH-OPF refers to the method of Kos et al. [40]. *L. fermentum* InaCC B1295 was inoculated as much as 0.5 mL into 50 mL MRSB and incubated at 37 ℃ for 20 hours. The cells were centrifuged at 4500 rpm for 15 minutes at 4 ℃ to obtain cell biomass. The cell biomass was washed twice with phosphate-buffered saline (PBS) pH 7.2 and resuspended in 60 mL of PBS. The cell suspension was mixed using a vortex for 10 seconds and evaluated at 0 and 5 hours of incubation at 37 ℃. 0.1 mL of the upper suspension was transferred

to a tube containing 3.9 mL PBS. The absorbance was measured using a spectrophotometer at an absorbance of 600 nm. The autoaggregation percentage is calculated using the following equation:

$$\text{Autoaggregation (\%)} = \left(1 - (A_t/A_o)\right) \times 100 \quad (1)$$

A_t is the absorbance at 5 hours; A_0 is the absorbance at 0 hours.

2.9. Coaggregation activity

Coaggregation of *L. fermentum* InaCC B1295 with or without CMF encapsulation of OPF was carried out according to the method of Kumar et al. [41]. *L. fermentum* InaCC B1295 with or without encapsulated CMFH-OPF or *Pediococcus pentosaceus* strain 2397 was inoculated 0.5 mL each into 50 mL MRSB medium. *Escherichia coli* FNCC-19 and *S. aureus* FNCC-15 were inoculated as much as 0.5 mL into 50 mL of NB medium. The bacterial coaggregation mixture was incubated at 37 ℃ for 20 hours. The cells were centrifuged at 4500 rpm for 15 minutes at 4 ℃ to obtain cell biomass. The cell biomass was washed twice with phosphate-buffered saline (PBS) pH 7.2, and each bacteria was resuspended in 60 mL of PBS. Each 2 mL cell suspension of each bacteria was mixed in pairs using a vortex for 10 seconds. 4 mL of each bacterial suspension was taken as a control. This suspension was incubated for 5 hours at 37 ℃. Absorbance measurements were carried out using a spectrophotometer at a wavelength of 600 nm at 0 and 5 hours of incubation time. The coaggregation percentage is calculated using the following equation:

$$\text{Coaggregation (\%)} = \frac{\frac{A_x + A_y}{2} - A(x+y)}{\frac{A_x + A_y}{2}} \times 100 \quad (2)$$

A_x is the absorbance of bacterial suspension x; A_y is the absorbance of bacterial suspension y, and $A(x + y)$ is the absorbance of a mixture of the suspensions of the two bacteria.

2.10. Cell surface hydrophobicity

CSH was determined according to the method of Xing et al. [42]. *L. fermentum* InaCC B1295 and *Pediococcus pentosaceus* strain 2397 cells encapsulated with oil palm leaf CMF hydrogel were grown in MRSB medium at 30 ℃ for 18 hours and then centrifuged at 10,000 rpm for 5 minutes. The cell pellet was rinsed twice with a phosphate urea magnesium (PUM) buffer at a pH of 7.1. The washed pellet (cell biomass) was resuspended in 60 mL of PUM buffer. The cell suspension was measured as 3.0 mL, and then 1.0 mL of xylene was added and mixed evenly using a vortex. Incubation was carried out at 30 ℃ for 10 minutes, then homogenized again using a vortex for 1 minute. Next, it was incubated at 30 ℃ for 1 hour for phase separation. The water phase was carefully removed, and the absorbance was measured at 600 nm. CSH is calculated using the following equation:

$$\text{Cell surface hydrophobicity (100\%)} = \left(1 - \frac{A_{after}}{A_{before}}\right) \times 100 \quad (3)$$

A_{after} is the absorbance of the initial suspension before adding xylene; A_{before} is the absorbance of the initial suspension after adding xylene.

2.11. Data analysis

Data on acid and bile tolerance, aggregation, and CSH were analyzed statistically using the t-test and presented as figures.

3. Results and discussion

3.1. Viability of Limosilactobacillus fermentum InaCC B1295 to acid and bile assay

The resistance of probiotics to acid is an important factor in their effectiveness and survival within the gastrointestinal tract. The gastrointestinal system, particularly the stomach, is a highly acidic environment due to gastric acid. The acid tolerance values of *Lactobacillus fermentum* InaCC B1295 cells encapsulated or without encapsulation with CMF hydrogel from OPF are presented in Table 1.

Table 1. Acid tolerance values of free cells and encapsulated cells of *Limosilactobacillus fermentum* InaCC B1295 without CMF hydrogel from OPF.

Treated cells	at pH 6.5 (log CFU/mL)	at pH 2 (log CFU/mL	Reduction (log CFU/mL)
Free cells	10.21 ± 0.56^{a}	6.19 ± 0.34^{b}	4.02 ± 0.32^{b}
Encapsulated cells	9.91 ± 0.73^{a}	9.83 ± 0.62^{a}	0.08 ± 0.01^{a}

[a,b] Means in the same column followed by different superscript letters indicate significant ($p < 0.05$).

The data in Table 1 show that free and encapsulated cells of LFB1295 were kept at pH 2, resembling the human stomach's pH, showing a significant decrease, especially in free cells. This finding is because LFB1295 free cells will come into direct contact with extreme pH without protection, which causes cell death. Meanwhile, the encapsulated cells contain protection in the form of CMF hydrogel from OPF, which protects the LFB1295 cells from extreme pH. One of the advantages of cellulose and its derivatives is that they do not dissolve at pH ≤ 5 [43]. Thus, LFB1295 cells remain encased in CMFH from OPF while passing through the stomach pH, which can reach pH 2.

Cellulose microfiber is derived from the primary constituent of plant cell walls, cellulose. Diverse techniques disintegrate cellulose into delicate fibers, yielding a material with distinctive characteristics and uses. The primary function of cellulose microfiber as an encapsulant is to protect the probiotics from adverse environmental factors such as acidic pH. This protective layer ensures the viability and effectiveness of the probiotics, ensuring their survival until they reach the intestines, where they can have beneficial effects. Cellulose microfibre enables the specific and regulated delivery of probiotics within the digestive system.

Furthermore, this controlled release method ensures an increasing release of probiotics in the gastrointestinal tract, enhancing their survivability and enabling consistent delivery of beneficial microbes to the intended location. According to Anselmo et al. [44], study has indicated that *Bacillus coagulans* encapsulated in one or two bilayers of chitosan/alginate reduced the bacteria in simulated gastric fluid by 4 log CFU/mL. Similar to observations by Charteris et al. [45] and Hansen et al. [46], who showed reductions of roughly 3 log CFU/mL for *Bifidobacterium adolescentis* subjected to simulated gastric juice (pH 2.0) for 2 to 3 h, the viable population for free *Bifidobacterium adolescentis* cells also decreased by 3.45 log CFU/mL. Additionally, probiotics from the genera Lactobacillus and

Bifidobacterium that were microencapsulated with alginate survived in MRS containing HCl more effectively than free probiotics [22].

LFB1295 must continuously travel through intestinal fluid and gastric fluid in order to colonize the intestinal tract (small and large intestine) successfully. We further investigated the protective role of OPF-derived CMFH on LFB1295's ongoing tolerance to bile salts. The resistance of probiotics to bile acids is an important factor in their ability to survive and function effectively in the gastrointestinal tract. The liver produces bile acids, which play a crucial role in the digestion and absorption of dietary fats. Probiotics encounter bile acids in the small intestine when ingested orally as supplements or through fermented foods. The bile tolerance values of *Limosilactobacillus fermentum* InaCC B1295 cells encapsulated or without encapsulation with CMFH from OPF are presented in Table 2.

Table 2. Bile tolerance values of free cells and encapsulated cells of *Limosilactobacillus fermentum* InaCC B1295 without CMFH from OPF.

Treated cells	MRS-THIO without ox gall (log CFU/mL)	MRS-THIO with ox gall 0.3% (log CFU/mL)	Reduction (log CFU/mL)
Free cells	9.96 ± 0.57^{a}	8.90 ± 0.63^{b}	1.06 ± 0.08^{b}
Encapsulated cells	10.01 ± 0.69^{a}	9.88 ± 0.39^{a}	0.13 ± 0.02^{a}

[a,b] Means in the same column followed by different superscript letters indicate significant ($p < 0.05$).

The data presented in Table 2 demonstrates that both free and encapsulated cells exhibited a reduction in cell count, with the free cells seeing the most significant decline of 1.06 log CFU/mL. The total number of encapsulated cells remained constant when cultivated in a medium containing 0.3% bile salt. The CMFH encapsulant from OPF protects cells from the adverse conditions of bile salts in the small intestine. Nevertheless, the number of cells tends to decrease slightly due to the small intestine's pH of around 6. This fact leads to the dissolution of cellulose and its derivatives, subsequently releasing the enclosed cells. Encapsulated cells are released into free cells in the colon when cellulose-based encapsulants and their derivatives solubilize at a pH ≥ 6 rather than below pH < 5 [43].

The physicochemical properties of encapsulation are crucial because apart from protecting the passage through acidic conditions in the stomach and bile in the small tract, cellulose-based encapsulants can also release cells in the colon to perform their therapeutic function. The sensitivity of bacteria to bile concentrations encountered in the human gastrointestinal tract has been reported by several authors. After being immersed in simulated intestinal fluid for five hours, the viable counts of free and encapsulated *Lactobacillus paracasei* revealed reductions of around 9.2 and 3.0 log CFU/mL, respectively. Studies have shown that encapsulating *Bacillus coagulans* in one or two bilayers of chitosan/alginate decreased the quantity of bacteria in bile salt by less than 2 log CFU/mL [44]. After 12 hours at 37 °C, viable cell counts of *B. adolescentis* decreased by 5 log CFU/mL at bile concentrations of 2% [6]. Following a 2-h incubation period at 37 °C with 0.5% (w/v) bile, *B. adolescentis* was reduced by approximately 2 log CFU/mL [46]. *Bifidobacterium lactis* and *B. adolescentis* were shown to be more susceptible to the harmful effects of bile salt in a study conducted by Ben et al. [47] on the survival of these two species following exposure to bile salt stress. When free probiotic bacteria were exposed to ox gall, viability was reduced by 6.51 log CFU/mL, whereas only 3.36 log CFU/mL was lost in microencapsulated strains [22].

3.2. Autoaggregation

The process by which microorganisms, like LAB, interact to form groups or aggregates is called autoaggregation. More massive agglomerations or coalitions can proliferate in this proximity by interactions among bacteria of similar species. LAB can utilize their autoaggregation capabilities in certain situations to enhance their ability to connect to their hosts. This result performs the role of a barrier against pathogenic bacteria entering the digestive system and promotes the attachment of LAB to the gastrointestinal tract. The autoaggregation values of *Limosilactobacillus fermentum* InaCC B1295 cells encapsulated or without encapsulation with CMF hydrogel from OPF are presented in Figure 2.

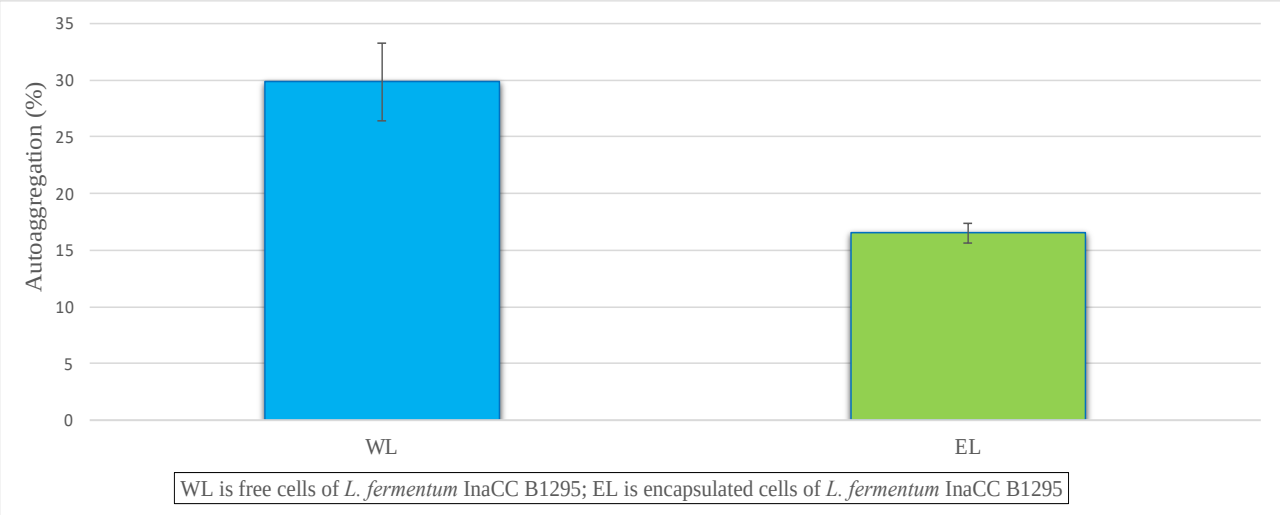

Figure 2. Autoaggregation values by free cells and encapsulated cells of *Limosilactobacillus fermentum* InaCC B1295 without CMF hydrogel from OPF.

The data in Figure 2 show that the autoaggregation value of LFB1295 cells encapsulated by CMFH from OPF is lower than that of free cells. This is because most LFB1295 cells are still covered in CMFH in digestive tract conditions with a pH < 6. CMFH covers the cell surface from bacteria, so receptors and chemical molecules in the cells will find it difficult to interact. Bacterial autoaggregation occurs because the adhesins found on the pili of bacterial cells bind to receptors on other bacterial cells [31].

Cellulose will prevent pili adhesins from recognizing receptor proteins on the cell surface, so adhesins on pili cannot mediate the attachment of bacteria to other bacteria. The present study found that the autoaggregation value of LFB1295 cells varied between 16.5 and 29.9%. The findings of this investigation closely resemble the autoaggregation values for *Lactobacillus plantarum*, *Lactobacillus paracasei*, and *Lactobacillus acidophilus* found in earlier investigations [30]. This range was less than the autoaggregation values of many strains of *Lactobacillus delbrueckii* subspecies *bulgaricus*, which ranged between 45.02 and 93.09% [48]. The cell-free autoaggregation value of LFB1295 was 29.9%, which was almost the same as the cell-free 22 Lactobacilli strains [31] but slightly higher with *Lactocaseibacillus rhamnosus* and *Lactiplantibacillus plantarum* [49]. The results showed that different Lactobacilli strains had different autoaggregation abilities. The cell membrane composition of each Lactobacillus species varies based on the presence of certain components such as protein,

glycoprotein, teichoic acid, and lipoteichoic acid [50]. The autoaggregation ability of Lactobacillus depends on the species. This statement is supported by Ekmekçi et al. [51], who reported autoaggregation values for several Lactobacillus species in the range of 21 to 97%.

3.3. Coaggregation

Probiotics can compete with dangerous bacteria for adhesion to the epithelial cells of the human digestive tract. Probiotics may function this way, preventing pathogenic bacteria from attaching to the sites required to cause an infection. The coaggregation values of *Limosilactobacillus fermentum* InaCC B1295 cells, either encapsulated or not using CMF hydrogel from OPF, are displayed in Figure 3.

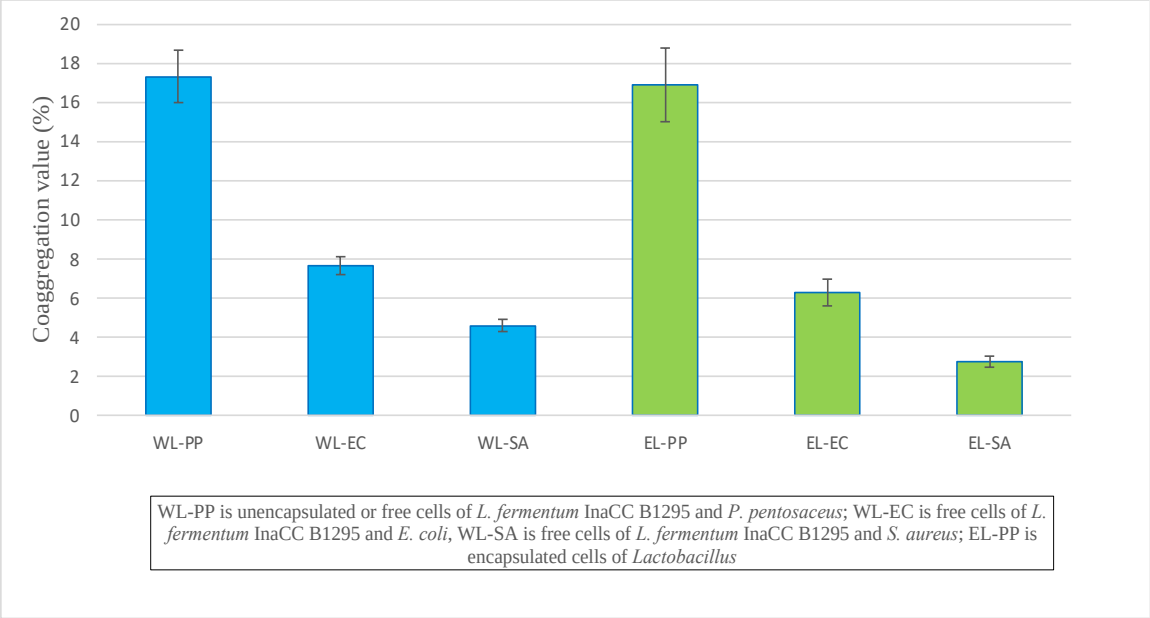

Figure 3. Coaggregation values by free cells and encapsulated cells of *Limosilactobacillus fermentum* InaCC B1295 without CMF hydrogel from OPF.

The coaggregation value of LFB1295-free cells was generally higher than that of cells encapsulated with CMFH from OPF. This is likely due to the encapsulated cells being slow to release from the encapsulant due to conditions that make them slow to aggregate with other bacteria. Free cells or cells from the encapsulant interact with other bacterial cells. The attachment of bacterial aggregates is influenced by complex interactions of components found on each bacterial cell surface [34]. Adhesins on encapsulated cell pili can still interact with protein receptors on non-encapsulated bacterial cells. The coaggregation ability of LFB1295, both free and encapsulated cells, with fellow LAB (*Pediocccus pentosaceus*) has a high value, namely 16.9–17.3%, while coaggregation with pathogens (*E. coli* and *S. aureus*) is only 2.8–7.7%. The low coaggregation ability against pathogens is caused by the presence of antimicrobials produced by LAB. The antimicrobial produced was a bacteriocin, which can inhibit the growth of pathogens [34]. These results align with the research of Panjaitan et al. [34], which conducted coaggregation tests on *L. fermentum* 2 BK 2–7 and *P. pentosaceus* 1 W2SR04 against *E. coli* ATCC 11230 with a coaggregation value of around 7%. The coaggregation ability of *Lactobacillus fermentum* InaCC B1295 cells with a range of 4.60–17.32 is

slightly lower than several previously reported Lactobacillus strains [30,52]. However, the coaggregation value of LB1295 cells was almost the same as that of the *Lactobacillus delbrueckii* subspecies *bulgaricus* strain 22 as reported by [48]. LFB1295 cells had a higher coaggregation value with *E. coli* than with *S. aureus*. This result contrasts the previous finding that *S. aureus*-isolated LABs from human breast milk had a better coaggregation value than *E. coli*-isolated LABs [53].

This ability is necessary for probiotics to form colonies and prevent pathogens from living in the digestive tract. The higher the coaggregation value of probiotics with LAB, the better it is for them to coexist in the digestive tract. Conversely, the lower the coaggregation value of probiotics with pathogens, the more the number of pathogens that will stick to the digestive tract is reduced, thereby reducing the potential for harmful interference from pathogens. The results of this research show that free cells and encapsulated cells of LFB1295 prevent the adhesion of *E. coli* to a greater extent than *S. aureus*. Enteropathogenic *E. coli* (EPEC) can cause gastroenteritis, whose symptoms involve diarrhea, nausea, vomiting, and fever. Some studies suggest that certain *E. coli* infections may play a role in developing intestinal bowel diseases, such as Crohn's disease and ulcerative colitis. Some other strains of *E. coli*, especially *E. coli* O157:H7, can cause hemolytic uremic syndrome (HUS), a condition that can occur in children and causes damage to the blood, kidneys, and nervous system [54,55]. *S. aureus* can produce enterotoxins, which can cause food poisoning. *Staphylococcus aureus* can also cause local infections in the digestive tract, such as abscesses or infections of the stomach and intestines. Symptoms can include abdominal pain, nausea, vomiting, and indigestion [56,57].

3.4. Hydrophobicity

Many molecules that enter the digestive system must interact with water to be digested, absorbed, and utilized by the body. The human digestive tract is a water-rich environment. Therefore, its hydrophobic nature can significantly impact a molecule's ability to function in the digestive tract. The CSH values of *L. fermentum* InaCC B1295 cells encapsulated or without encapsulation with CMF hydrogel from OPF are presented in Figure 4.

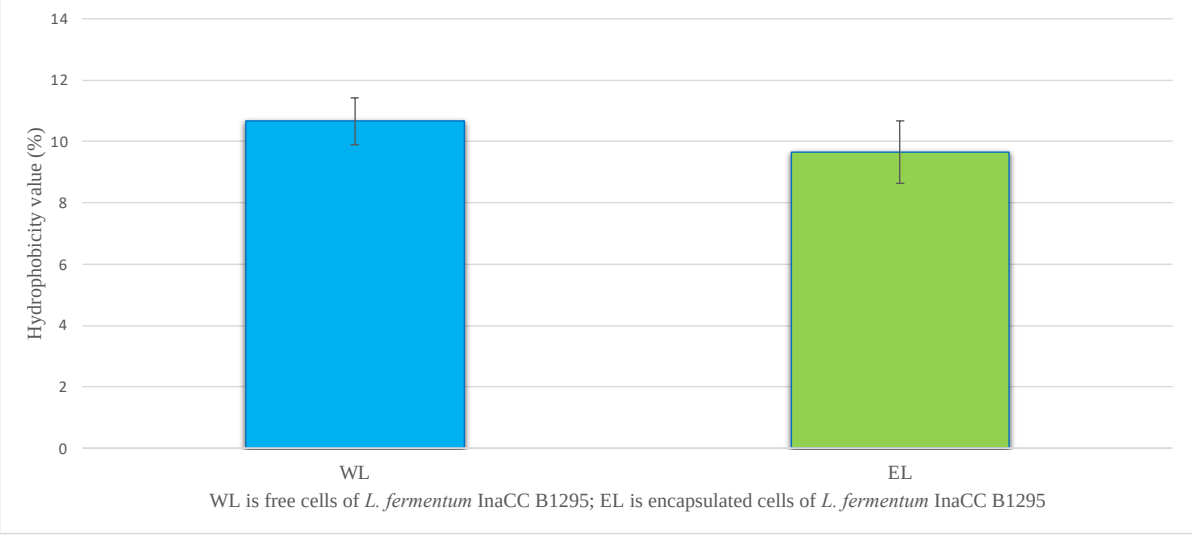

Figure 4. Cell surface hydrophobicity values by free cells and encapsulated cells of *Limosilactobacillus fermentum* InaCC B1295 without CMF hydrogel from OPF.

Figure 4 shows that LFB1295 cells encapsulated with CMFH from OPF have lower CSH when compared with free cells. This is because the encapsulated cells will affect the bacteria's ability to interact with water. The cell surface structure changes due to the coating of CMF, resulting in properties and capabilities that are different from bacterial cells without encapsulation.

CMF can form a hydrophobic layer around the surface of bacterial cells, resulting in an increase in CSH of around 4–6% in the encapsulated cells. A cellulose capsule is a hydrophobic layer surrounding bacterial cells [58]. CMF will interact with hydrocarbon solvents around bacterial cells by binding to the hydroxyl groups in CMF through hydrogen bonds. Jiang et al. [59] stated that the interaction between cellulose and hydrocarbon solvents is due to van der Waals forces. Cellulose has a non-polar part in each amorphous structure that can bind with hydrocarbon solvents. This results in water being unable to form hydrogen bonds on the surface of bacterial cells. The genus of the bacteria also influences CSH. The difference in CSH values of these bacteria is caused by the composition of lipids and proteins found on the surface of bacterial cells. These results align with research by Panjaitan et al. [34], who reported that *P. pentosaceus* 1 W2SR04 and *L. fermentum* 2 BK 2–7 have a difference in CSH values of 8.69%. The autoaggregation value of encapsulated and free LFB1295 cells was 9.7–10.7% in this study, lower than the autoaggregation values of several strains of *Lactobaillus plantaram, L. casei, L. rhamnosus, L. paracasei,* and *L. acidophilus* [30,52] Massounga et al. [8] also reported that the cell surface hydrophobicity of *L. acidophilus* without encapsulation resulted in a lower hydrophobicity value of 21% compared to 48% for encapsulated cells with newly designed biopolymeric-based encapsulates.

Bacteria with high lipid content in their cell membranes can form thicker, more complex biofilm layers. Lipids and a biofilm layer on the cell surface will prevent bacteria from interacting with water, thus increasing their hydrophobic properties. This biofilm layer has several compositions, such as hydrophobic polysaccharides and proteins [60]. Hydrophobicity can influence the ability of probiotics to adhere to the mucosal surfaces of the gastrointestinal tract. Probiotics that exhibit hydrophobic properties may adhere more effectively to the mucosa, allowing them to establish a more substantial presence and exert their beneficial effects more efficiently [61]. Hydrophobicity is often associated with forming biofilms, communities of microorganisms encased in a protective matrix. In probiotics, biofilm formation can contribute to their ability to colonize and persist in the gut, enhancing their overall efficacy. Differences in cell wall composition in each bacteria greatly influence their hydrophobicity. Bacterial cell wall components play a role in attachment to host cells, which forms hydrophobic interactions [36,62].

4. Conclusions

The study demonstrates that cells encapsulated in OPF CMFH are more resistant to bile salts and stomach acid than cells without OPF. This is supported by the significant decrease in free cell viability observed in the latter group. Conversely, LFB1295 free cells exhibited higher levels of autoaggregation, cell surface hydrophobicity, and coaggregation than OPF cells encapsulated in CMGH. Unlike coaggregation with pathogenic bacteria, especially harmful *S. aureus* and *E. coli*, free and encapsulated cells generally had higher coaggregation values than fellow LAB (*P. pentosaceus*). These results suggest that free cells or cells encapsulated in CMFH from OPF can be confidently considered probiotics due to their excellent bile and acid salt tolerance, autoaggregation, coaggregation, and cell surface hydrophobicity.

Use of AI tools declaration

The authors declare that they have not used artificial intelligence (AI) tools in the creation of this article.

Acknowledgments

The authors appreciate the research grant from the Ministry of Education, Culture, Research and Technology of the Republic of Indonesia.

Conflict of interest

The authors declare that there are no conflicts of interest.

Author contributions

Research on the main theme was initiated by UP, who also oversaw various stages of the project. UP, YY, and AS carried out the following research stages in this study: designing the experiments and supervising several phases of the work. UP, YY, AS, and ER are the main contributors to the research and writing of the article. This experiment was planned by UP and EMR, who also proofread the article. Article writing and editing were done in collaboration with UP and EMR.

References

1. Diez-Gutiérrez L, San Vicente L, Luis LJ, et al. (2020) Gamma-aminobutyric acid and probiotics: Multiple health benefits and their future in the global functional food and nutraceuticals market. *J Funct Foods* 64: 1–14. https://doi.org/10.1016/j.jff.2019.103669
2. Salminen S, van Loveren H (2012) Probiotics and prebiotics: Health claim substantiation. *Microb Ecol Health Dis* 23: 0–3. https://doi.org/10.3402/mehd.v23i0.18568
3. Butel M (2014) Probiotics, gut microbiota and health. *Med Mal Infect* 44: 1–8. https://doi.org/10.1016/j.medmal.2013.10.002
4. Martín MJ, Lara-Villoslada F, Ruiz MA, et al. (2015) Microencapsulation of bacteria: A review of different technologies and their impact on the probiotic effects. *Innovative Food Sci Emerging Technol* 27: 15–25. https://doi.org/10.1016/j.ifset.2014.09.010
5. Gbassi GK, Vandamme T (2012) Probiotic encapsulation technology: From microencapsulation to release into the gut. *Pharmaceutics* 4: 149–163. https://doi.org/10.3390/pharmaceutics4010149
6. Annan NT, Borza AD, Hansen LT (2008) Encapsulation in alginate-coated gelatin microspheres improves survival of the probiotic *Bifidobacterium adolescentis* 15703T during exposure to simulated gastrointestinal conditions. *Food Res Int* 41: 184–193. https://doi.org/10.1016/j.foodres.2007.11.001
7. Rodrigues FJ, Cedran MF, Bicas JL, et al. (2020) Encapsulated probiotic cells : Relevant techniques, natural sources as encapsulating materials and food applications—A narrative review. *Food Res Int* 137: 109682. https://doi.org/10.1016/j.foodres.2020.109682
8. Massounga BAF, Li X, Liu L (2021) Physicochemical and functional characterization of newly designed biopolymeric-based encapsulates with probiotic culture and Charantin. *Foods* 10: 2677. https://doi.org/10.3390/foods10112677

9. Setiarto RHB, Kusumaningrum HD, Jenie BSLJ, et al. (2018) Technology development of probiotic bacteria microencapsulation and it benefit for healthy. *J Vet* 19: 574–589. https://doi.org/10.19087/jveteriner.2018.19.4.574
10. Gunawan C, Asben A, Anggraini T, et al. (2021) Production and characterization of microcrystalline cellulose from oil palm trunk waste (*Elaeis guineensis* jacq.) from plantation replanting. *J Penelitian Kelapa Sawit* 29: 137–146. https://doi.org/10.22302/iopri.jur.jpks.v29i3.139
11. Directorate General of Estates (2022) Tree Crop Estate Statistics of Indonesia 2018–2020. Available from: www.ditjenbun.pertanian.go.id.
12. Pato U, Yusmarini, Riftyan E, et al. (2023) Physicochemical characteristics of oil palm frond and application of CMF Hydrogel as a natural encapsulant for probiotic. *IOP Conf Ser: Earth Environ Sci* 1228: 012002. https://doi.org/10.1088/1755-1315/1228/1/012002
13. Hosono A, Wardoyo R, Otani H (1989) Microbial flora in dadih, a traditional fermented milk in Indonesia. *J Technol* 22: 20–24
14. Shokryazdan P, Sieo CC, Kalavathy R, et al. (2014) Probiotic potential of Lactobacillus strains with antimicrobial activity against some human pathogenic strains. *BioMed Res Int* 2014: 927268. https://doi.org/10.1155/2014/927268
15. Khalesi S, Bellissimo N, Vandelanotte C, et al. (2019) A review of probiotic supplementation in healthy adults : helpful or hype?. *Eur J Clin Nutr* 73: 24–37. https://doi.org/10.1038/s41430-018-0135-9
16. Mazaya B, Saafan AE, Aziz M (2016) Antibacterial activity of Lactobacilli Against *Salmonella typhi*. *Middle-East J Sci Res* 24: 1887–1898. https://doi.org/10.5829/idosi.mejsr.2016.24.06.10174
17. Plant L, Lam C, Conway PL, et al. (2003) Gastrointestinal microbial community shifts observed following oral administration of a Lactobacillus fermentum strain to mice. *FEMS Microbiol Ecol* 43: 133–140. https://doi.org/10.1016/S0168-6496(02)00374-4
18. Pato U, Riftyan E, Rossi E, et al. (2022) Probiotic Properties of *Lactobacillus fermentum* InaCC B1295 Encapsulated by Cellulose Microfiber from Oil Palm Empty Fruit Bunches. *Fermentation* 8: 602. https://doi.org/10.3390/fermentation8110602
19. Xie J, Yao M, Lu Y, et al. (2021) Impact of encapsulating a probiotic (*Pediococcus pentosaceus* Li05) within gastro-responsive microgels on Clostridium difficile infections. *Food Funct* 12: 3180–3190. https://doi.org/10.1039/d0fo03235b
20. Pato U (2003) Bile and acid tolerance of Lactic Acid Bacteria isolated from Dadih and their antimutagenicity against mutagenic heated Tauco. *Asian-Australas J Anim Sci* 16: 1680–1685. https://doi.org/10.5713/ajas.2003.1680
21. Hu PL, Yuan YH, Yue TL, et al. (2018) Bile acid patterns in commercially available oxgall powders used for the evaluation of the bile tolerance ability of potential probiotics. *PLOS ONE* 13: e0192964. https://doi.org/10.1371/journal.pone.0192964
22. Ding WK, Shah NP (2007) Acid, bile, and heat tolerance of free and microencapsulated probiotic bacteria. *J Food Sci* 72: M446–M450. https://doi.org/10.1111/j.1750-3841.2007.00565.x
23. Pato U (2012) Probotics: Prospects and implementation in the field of functional foods and health. Universitas Riau, Pekanbaru.
24. Pourramezan Z, Oloomi M, Kasra-Kermanshahi R (2020) Antioxidant and anticancer activities of *Lactobacillus Hilgardii* strain AG12a. *Int J Prev Med* 11: 132. https://doi.org/10.4103/ijpvm.IJPVM_307_19

25. Pato U, Hosono A (1999) Binding of cholesterol to the cells and peptidoglycan of *Lactobacillus gasseri. Milchwissenschaft* 54: 495–498.
26. Pato U, Ali M, Parlindungan AK (2005) Taurocholate deconjugation and cholesterol binding by indigenous Dadih Lactic Acid Bacteria. *Hayati* 12: 103–107.
27. Widodo W, Fanani TH, Fahreza MI et al. (2021) Cholesterol assimilation of two probiotic strains of *Lactobacillus casei* used as dairy starter cultures. *Appl Food Biotechnol* 8: 103–112. https://doi.org/10.22037/afb.v8i2.30661
28. Liu Y, Wang J, Wu C (2022) Modulation of gut microbiota and immune system by probiotics. *Front Nutr* 8: 634897. https://doi.org/10.3389/fnut.2021.634897
29. Hu S, Wang L, Jiang Z (2017) Dietary additive probiotics modulation of the intestinal microbiota. *Protein Pept Lett* 24: 382–387. https://doi.org/10.2174/0929866524666170223143615
30. Abdulla AA, Abed TA, Saeed AM (2014) Adhesion, autoaggregation and hydrophobicity of six Lactobacillus strains. *Microbiol Res J Int* 4: 381–391. https://doi.org/10.9734/BMRJ/2014/6462
31. Trunk T, Khalil HS, Leo JC (2018) Bacterial autoaggregation. *AIMS Microbiol* 4: 140–164. https://doi.org/10.3934/microbiol.2018.1.140
32. Abushelaibi A, Al-Mahadin S, El-Tarabily K, et al. (2017) Characterization of potential probiotic lactic acid bacteria isolated from camel milk. *LWT-Food Sci Technol* 79: 316–325. https://doi.org/10.1016/j.lwt.2017.01.041
33. Krausova G, Hyrslova I, Hynstova I (2019) In vitro evaluation of adhesion capacity, hydrophobicity, and auto-aggregation of newly isolated potential probiotic strains. *Fermentation* 5: 1–11. https://doi.org/10.3390/fermentation5040100
34. Panjaitan R, Nuraida L, Dewanti-Hariyadi R (2018) Selection of lactic acid bacteria isolated from tempe and tape as probiotic candidates. *J Teknologi dan Industri Pangan* 29: 175–184. https://doi.org/10.6066/jtip.2018.29.2.175
35. Affhan S, Dachang W, Xin Y, et al. (2015) Lactic acid bacteria protect human intestinal epithelial cells from *Staphylococcus aureus* and *Pseudomonas aeruginosa* infections. *Genet Mol Res* 14: 17044–17058. https://doi.org/10.4238/2015.December.16.5
36. Das S, Vishakha K, Banerjee S, et al. (2022) A novel probiotic strain of *Lactobacillus fermentum* TIU19 isolated from Haria beer showing both in vitro antibacterial and antibiofilm properties upon two multi resistant uro-pathogen strains. *Curr Res Microb Sci* 3: 1–9. https://doi.org/10.1016/j.crmicr.2022.100150
37. Pato U, Ayu DF, Riftyan E, et al. (2022) Cellulose microfiber encapsulated probiotic: viability, acid and bile tolerance during storage at different temperature. *Emerging Sci J* 6: 106–117. https://doi.org/10.28991/ESJ-2022-06-01-08
38. Shehata MG, Sohaimy SA, El, El-sahn MA, et al. (2016) Screening of isolated potential probiotic lactic acid bacteria for cholesterol lowering property and bile salt hydrolase activity. *Ann Agric Sci* 61: 65–75. https://doi.org/10.1016/j.aoas.2016.03.001
39. Baig MA, Turner MS, Liu SQ, et al. (2021) Potential probiotic *Pediococcus pentosaceus* M41 modulates its proteome differentially for tolerances against heat, cold, acid, and bile stresses. *Front Microbiol* 12: 1–12. https://doi.org/10.3389/fmicb.2021.731410
40. Kos B, Suskovic J, Vukovic S, et al. (2003) Adhesion and aggregation ability of probiotic strain *Lactobacillus acidophilus* M92. *J Appl Microbiol* 94: 981–987.

41. Kumar R, Bansal P, Singh J, et al. (2020) Aggregation, adhesion and efficacy studies of probiotic candidate Pediococcus acidilactici NCDC 252 : A strain of dairy origin. *World J Microbiol Biotechnol* 8: 1–15. https://doi.org/10.1007/s11274-019-2785-8
42. Xing Z, Tang W, Geng W, et al. (2017) In vitro and in vivo evaluation of the probiotic attributes of *Lactobacillus kefiranofaciens* XL10 isolated from Tibetan kefir grain. *Appl Microbiol Biotechnol* 101: 2467–2477. https://doi.org/10.1007/s00253-016-7956-z
43. Koh WY, Lim XX, Tan T, et al. (2022) Encapsulated probiotics: potential techniques and coating materials for non-dairy food applications. *Appl Sci* 12: 10005. https://doi.org/10.3390/app121910005
44. Anselmo AC, Mchugh KJ, Webster J, et al. (2016) Layer-by-layer encapsulation of probiotics for delivery to the microbiome. *Adv Mater* 28: 9486–9490. https://doi.org/10.1002/adma.201603270
45. Charteris WP, Kelly PM, Morelli L, et al. (1998) Development and application of an in vitro methodology to determine the transit tolerance of potentially probiotic Lactobacillus and Bifidobacterium species in the upper human gastrointestinal tract. *Fuller* 759–768.
46. Hansen LT, Allan-Wojtas PM, Jin YL, et al. (2002) Survival of Ca-alginate microencapsulated Bifidobacterium spp. in milk and simulated gastrointestinal conditions. *Food Microbiol* 19: 35–45. https://doi.org/10.1006/fmic.2001.0452
47. Amor KB, Breeuwer P, Verbaarschot P, et al. (2002) Multiparametric flow cytometry and cell sorting for the assessment of viable, injured, and dead Bifidobacterium cells during bile salt stress. *Appl Environ Microbiol* 68: 5209–5216. https://doi.org/10.1128/AEM.68.11.5209
48. Aslim B, Onal D, Beyatli Y (2007) Factors influencing autoaggregation and aggregation of *Lactobacillus delbrueckii* subsp. *bulgaricus* isolated from handmade yoghurt. *J Food Prot* 70: 223–227. https://doi.org/10.4315/0362-028X-70.1.223
49. Rojek AZ, Kośmider A, Stępień K, et al. (2022) Adhesion and aggregation properties of Lactobacillaceae strains as protection ways against enteropathogenic bacteria. *Arch Microbiol* 204: 1–13. https://doi.org/10.1007/s00203-022-02889-8
50. Nwoko EQA, Okeke IN (2021) Bacteria autoaggregation: how and why bacteria stick together. *Biochem Soc Trans* 1: 1147–1157. https://doi.org/https://doi.org/10.1042/BST20200718
51. Havva E, Belma A, Derya DO (2009) Some factors affecting the autoaggregation ability of vaginal lactobacilli isolated from turkish women. *Arch Biol Sci* 61: 407–412. https://doi.org/10.2298/ABS0903407E
52. Yu H, Wu Z, Guo B, et al. (2013) Aggregation and adhesion properties of 22 Lactobacillus strains. *J Dairy Sci* 96: 4252–4257. https://doi.org/10.3168/jds.2013-6547
53. Kusmiyati N, Wicaksono ST, Sukarno AS (2022) Isolation and characterization of probiotic lactic acid bacteria from human breast milk. *Nova Biotechnol Chim* 21: e1053. https://doi.org/10.36547/nbc.1053
54. Ingerson M, Reid A (2011) *E. coli*: good, bad, & deadly. Conference: American Academy of Microbiology FAQ, 1–17. Available from: https://www.researchgate.net/publication/281629337_E_coli_Good_bad_deadly/citations.
55. Torso LM, Voorhees RE, Forest SA, et al. (2015) *Escherichia coli* O157:H7 outbreak associated with restaurant beef grinding. *J Food Prot* 78: 1272–1279. https://doi.org/10.4315/0362-028X.JFP-14-545
56. Goss CH, Muhlebach MS (2011) *Staphylococcus aureus* and MRSA in cystic fibrosis. *J Cystic Fibrosis* 10: 298–306. https://doi.org/10.1016/j.jcf.2011.06.002

57. Todar K (2012) *Staphylococcus aureus* and Staphylococcal Disease. *Todar's Online Textbook of Bacteriology.* Available from: https://textbookofbacteriology.net/staph.html
58. Pepicelli M, Binelli MR, Studart AR, et al. (2021) Self-grown bacterial cellulose capsules made through emulsion templating. *ACS Biomater Sci Eng* 7: 3221–3228. https://doi.org/10.1021/acsbiomaterials.1c00399
59. Seddiqi H, Oliaei E, Honarkar H, et al. (2021) Cellulose and its derivatives: Towards biomedical applications. *Cellulose* 28: 1893–1931. https://doi.org/10.1007/s10570-020-03674-w
60. Jiang Y, Geng M, Bai L (2020) Targeting biofilms therapy: current research strategies and development hurdles. *Microorganisms* 8: 1222. https://doi.org/10.3390/microorganisms8081222
61. Danchik C, Casadevall A (2021) Role of cell surface hydrophobicity in the pathogenesis of medically-significant fungi. *Front Cell Infect Microbiol* 10: 1–7. https://doi.org/10.3389/fcimb.2020.594973
62. Abitbol T, Rivkin A, Cao Y, et al. (2016) Nanocellulose, a tiny fiber with huge applications. *Curr Opin Biotechnol* 39: 76–88. https://doi.org/10.1016/j.copbio.2016.01.002

AIMS Press