Abstract:

The present study was undertaken to evaluate the properties of Lactobacillus plantarum T23/3 isolated from the feces of healthy elderly volunteer. The L. plantarum T23/3 showed better tolerance property to bile acid, simulated gastric fluid (SGF) and simulated intestinal fluid (SIF), and exhibited antimicrobial activity against tested enteropathogens. It formed macroscopic granules with surface hydrophobicity and co-aggregated with tested microbial strain. It was resistant to some antibiotics used for treatment of diarrhea so there is possibility to co-administer L. plantarum as probiotic with antidiarrheal therapy. Furthermore, adhesion and significant potential for decreasing adhesion of pathogens (E. coli 2601 and V. cholera 3194) to Caco-2 cells were observed in these experiments. The stability profile of L. plantarum T23/3 reflected viability (upto 12 months) in enteric coated capsules containing lactose. While the freeze-dried lactobacilli loaded into the Eudragit®L-100 coated capsule did not release the probiotic content in SGF pH 1.2 over 2 h but dissolved, released the L. plantarum 23/3 instantly in the SIF at pH 7.4. Our study provides evidence that L. plantarum T23/3 has a promising future to protect humans from gastrointestinal infections and affirms to serve as a probiotic.

Keywords: Probiotic; Antimicrobial activity; Adhesion; Survival; Stability
Introduction

The World Health Organization has currently advocated implementation of alternative strategies for controlling gastrointestinal problems by exploiting the prophylactic and therapeutic potential of probiotic bacteria [1]. Gastrointestinal microflora is been recognized as one of the factors that influence human health [2]. This microflora has metabolic, trophic, and protective functions, and the exogenous administration of probiotics can modify these functions to protect individuals against pathological gastrointestinal conditions. Probiotics are live microorganisms that when administered in adequate amounts confer a health benefit to the host [3]. The most frequently used strains include members of the genus Lactobacillus. Their presence in human flora and long history of use in foods and dairy products without significant complications has led to the conclusion that they are safe for human consumption as such, many strains have obtained generally recognized as safe (GRAS) status [4]. The beneficial strategies of lactobacilli include direct effects upon the host immunomodulation, reduction of infection risks by strengthening of the barrier function of the intestinal epithelial monolayer, by stimulation of mucin secretion or enhancement of tight-junction functioning [5]. Alternatively, probiotics may directly affect the clearance of pathogens by competitive exclusion for example by synthesizing antimicrobial substances such as bacteriocins or lactic acid [6, 7]. Although many potential probiotic strains are currently available for commercial use in the form of fermented foods or pure cultures in powder, tablet or capsule form, many of these bacteria must survive passage through the upper gastrointestinal tract and arrive alive and bioactive at the site of action, preserving their ability to function in the gut environment. Other requirements include uniform distribution of the probiotics in the formula, as well as their stability during product processing, distribution and storage [8].

The objective of the present study was to characterize the potential probiotic properties of Lactobacillus plantarum T23/3 (L. plantarum T23/3) for its preventive and therapeutic action in gastrointestinal infections. An alternative method was developed for the enteric coating capsules containing viable lactobacilli. We also evaluated the survival of cells after freeze-dried processing, under simulated gastrointestinal conditions and during storage at 4°C over 12 months of the encapsulated cells.

Materials and Methods

Bacterial strains and growth condition

The L. plantarum T23/3 strain used in this study was isolated from feces of healthy elderly volunteers who had not taken antibiotics and had no recent history of gastrointestinal complaints for at least 3 months prior to the sampling date. This evidence proved that the volunteers have good microflora in the gut. The identity of lactobacilli was established at the species level by examining representative isolates for utilization of 49 carbohydrates using the API 50 CHL strips (API 50 systems, bioMérieux, France) according to the manufacturer instruction. The selected isolate was stored at -80°C in de Man Rogosa Sharpe broth (MRS, Difco, USA), supplemented with 20% (v/v) glycerol until used. For routine analysis the isolate was sub-cultured twice in MRS broth, each for 24 h at 37°C. The potential pathogenic test strains of clinical origin used for this study were: Escherichia coli (2601), Salmonella typhimurium (1756), Shigella sonnei (1549), Vibrio cholera (3194) and Staphylococcus aureus (1730). They were kindly provided by the Department of Pathology, Faculty of Medicine, Prince of Songkla University. Gastrointestinal pathogens were routinely grown in Brain Heart Infusion (BHI, Difco, USA) at 37°C for 18 h.

Identification of lactobacillus strain by 16S rRNA gene sequencing

The identification of lactobacilli was carried out using the protocol of Kaewsrichan et al. [9]. The primers LacAll-F(5'-TGCTTAATACATGCAAGTC-3') and LacAll-R(5'-CCTTGTACGACTTC ACC-3') were used to amplify nearly the full length of the 16S rRNA gene, corresponding to the conserved 16S rRNA gene regions of L. plantarum strain. PCR amplification was performed with a Perkin-Elmer DNA Thermal Cycler 9600.
programmed for 35 cycles, comprising denaturation at 94°C for 45 s, annealing at 50°C for 1 min and elongation at 72°C for 2 min, followed by a final extension at 72°C for 7 min. An amplicon of about 1500 bp in size was excised from a 1% agarose gel after electrophoresis, purified using the GFX™ Purification Kit (Amersham Biosciences, USA), and cloned into pGEM-T Easy using the procedures recommended by the manufacturer (Promega, USA). The 16S rRNA gene was sequenced directly using the recombinant plasmids as the DNA template, an ABI Prism Dye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, USA), T7 and SP6 universal primers, and primers designed by a walking strategy. Analysis of the alignment and homology for the sequences obtained was performed by BIOEDIT and BLAST programs. The obtained sequence was analyzed for its homology by comparing to the sequence reported in NCBI database.

**Antimicrobial activity**

The inhibitory activity of *L. plantarum* T23/3 against potential bacterial pathogens was measured by an agar overlay diffusion method [10]. Lactobacilli culture pellets were collected by centrifugation (5,000 rpm, 10 min, 4°C). The cell pellet of this strain were adjusted with phosphate buffered saline (PBS, pH 7.4, Difco, USA) to give turbidity equivalent to 0.5 McFarland standard corresponding to approximately 10^8 CFU/mL. Ten μL of lactobacilli cell pellet was spotted onto Brain Heart Infusion (BHI, Difco, USA) agar plates containing 1% glucose using a template guide and incubated anaerobically at 37°C for 4 days. Four mL of pathogens inoculum (10^8 CFU/mL) in soft agar medium was overlain on the top of the plates of lactobacilli. After incubation at 37°C for 18 h, the plates were observed for clear zones of inhibition around lactobacilli colonies with an antibiotic zone reader.

**Susceptibility to antibiotics**

The antibiotic susceptibility testing of *L. plantarum* T23/3 was determined by an agar diffusion method. The antibiotic discs (Oxoid, UK) were ampicillin (30 μg), chloramphenicol (30 μg), doxycycline (30 μg), erythromycin (15 μg), neomycin (30 μg), penicillin (10 units) and tetracycline (30 μg). The cell pellet of this strain was resuspended in PBS to a final concentration of 10^8 CFU/mL. The adjusted cultures were spread with a sterile cotton swab onto the surface of MRS agar plates. The antibiotics discs were placed onto the agar surface. After incubation under anaerobic condition at 37°C for 24 h, the plates were observed for a clear zone of inhibition around antibiotic discs with an antibiotic zone reader.

**Upper gastrointestinal transit tolerance assay**

The tolerance of probiotic stains to simulated gastric and small intestinal transit was determined following the method of Huang and Adams [11]. The cell pellet of *L. plantarum* T23/3 was resuspended in PBS to a final concentration of 10^8 CFU/mL. One mL of this suspension was added to 9 mL simulated gastric fluid (SGF) (containing pepsin (1:10000, ICN, Sigma, USA) in PBS to a final concentration of 3 g/L and adjusted to pH 2.0, 3.0, and 4.0) and simulated intestinal fluid (SIF) (containing pancreatin USP in PBS to a final concentration of 1 g/L and adjusting the pH to 8.0) before incubating under anaerobic condition at 37°C. Resistance was assessed in terms of viable colony counts and enumerated on MRS agar plates by drop plate technique after incubation at 37°C for 1, 2 and 3 h with SGF, and 1, 2, 3 and 4 h with SIF, to reflect the transit time in the stomach and small intestine, respectively.

**Bile tolerance assay**

The cell pellet of *L. plantarum* T23/3 was resuspended in PBS to a final concentration of 10^8 CFU/mL. One mL was then added to 9 mL PBS containing 0.3% w/v oxgall (Sigma, USA) and incubated under anaerobic conditions for 1, 2, 3 and 4 h at 37°C. Tolerance was assessed in terms of the viable colony counts enumerated on MRS agar plates.

**Autoaggregation test**

The cell pellet of *L. plantarum* T23/3 was resuspended and dispersed in PBS to a final
concentration of $10^8$ CFU/mL. One drop was placed on a glass slide and examined microscopically. Autoaggregation was determined as the ability to form aggregates within 2 min. *Lactobacillus cellobiosus* T13/5 that had also been isolated from the feces of a healthy elderly human was used as a negative control [12].

**Surface hydrophobicity**

Surface hydrophobicity was studied using the salt aggregation test (SAT). The cell pellet of *L. plantarum* T23/3 was resuspended in 20 mM of sodium phosphate buffer (pH 6.8) (Merck, Germany) to a final concentration of $10^7$ CFU/mL. Solutions (500 µL) of ammonium sulfate (Merck, Germany) (0.5, 1.5, 2.0 and 4.0 mol/L) were mixed with an equal volume of cell suspension on a glass slide and examined microscopically. The lowest concentration of ammonium sulfate that caused the bacteria to aggregate was defined as the SAT value [12].

**Co-aggregation assay of pathogens with lactobacilli**

The cell pellet of *L. plantarum* T23/3 and indicator pathogens (*E. coli* 2601, *S. typhimurium* 1756, *S. sonnei* 1549, *V. cholera* 3194 and *S. aureus* 1730) were resuspended in PBS, each to a final concentration of $10^8$ CFU/mL. Aliquots (500 µL) of the lactobacilli isolate were mixed with an equal volume of each indicator pathogen. The co-cultures were shaken in anaerobic conditions at 37°C, 100 rpm for 4 h. After incubation, the suspensions were smeared on a glass slide, Gram-stained and observed microscopically. A co-aggregation assay was positive as *L. plantarum* T23/3 formed aggregates with the other pathogenic [12].

**In vitro adherence assay**

A monolayer of enterocyte like Caco-2 cells (ATCC HTB-37, Rockville, USA) was used to determine the adhesion of lactobacilli. Cell growth and adherence assay were performed as previously described by Jankowska et al. [13]. Caco-2 cells were routinely grown in Eagle’s minimal essential medium (MEM, Gibco, USA) supplemented with 15% (v/v) fetal bovine serum (FBS; Gibco, USA), antibiotics (100 U/mL penicillin and 100 mg/mL streptomycin; Gibco, USA). Monolayers were prepared in 12-well tissue culture plates (Corning, USA) seeded (1 mL) at a concentration of $4.5 \times 10^5$ cells/well into the wells and incubated at 37°C in 5% CO$_2$ for 15 days with the culture medium changed daily.

The cell pellet of *L. plantarum* T23/3 was washed twice with PBS and resuspended in complete MEM without antibiotics to a final concentration of $10^7$ CFU/mL. For the adherence assay, Caco-2 monolayers in the wells of the tissue culture plates were washed twice with PBS and pre-incubated with 500 µL complete medium without antibiotics for at least 30 min before inoculation with the lactobacilli isolate. A bacterial MEM suspension (500 µL) was transferred onto the Caco-2 monolayers. The plate was incubated at 37°C in 5% CO$_2$ for 1 h. After incubation, the wells were washed four times with PBS to release unbound bacteria. The washed monolayer was then treated with 1 mL of 0.05% Triton X-100 and incubated at 37°C for 5 min to lyse the cells. The adhesion of the lactobacilli strain to Caco-2 cells was expressed as a percentage of the viable bacteria compared to their initial population.

**Inhibition of pathogen adhesion to Caco-2 cells**

To study the effect of lactobacilli on the binding of pathogens to enterocyte-like Caco-2 cells monolayers. Caco-2 cells were prepared as described before. The cell pellet of *L. plantarum* T23/3 and pathogens (*E. coli* 2601 and *V. cholera* 3194) were resuspended in MEM without antibiotic to provide $10^7$ CFU/mL of each culture. Aliquots (500 µL) of the lactobacilli strain and equal volumes of each indicator pathogens were added in a Caco-2 monolayer well plate. After incubation at 37°C for 1 h. Caco-2 cells were washed and the adhered pathogen populations were enumerated.

**Preparation of lactobacilli enteric coated capsules**

The cell pellet of *L. plantarum* T23/3 was resuspended in 8% lactose (Fluka, Germany). The suspensions obtained with a cell density of about $10^8$ CFU/mL were frozen overnight at -20°C followed by freeze-drying at -40°C for 2 h, then the sample was sublimed for 20 h at -10°C. In the final step the sample
temperature was raised to 25°C for 8 h to obtain a lyophilized powder (Dura-Stop and Dura-Dry, FTS system, USA). The lyophilized powder containing 10^8 cells of lactobacilli was filled into each hard gelatin capsule (size No. 1). Each batch of the capsules (5,000 capsules) was coated with 500-mL coating solution containing 12% Eudragit®L-100, 3% propylene glycol, 1% polysorbate 80, 45% ethyl alcohol and 39% acetone by a spray gun with nozzle diameter of 1.0 mm (Walther Pilot, Germany) at a flow rate of 3 mL/min. The coating process was carried out using conventional coating pan. The film was allowed to dry with the help of a dryer with an inlet temperature of 35-40°C for 10 min. The capsules were kept in airtight light resistant bottles containing silica-gel desiccants and stored at 4°C in the refrigerator. Viability of lactobacilli was determined at defined time intervals throughout a storage period for 12 months.

Viability of lactobacilli

The number of viable lactobacilli before and after freeze-drying and storage was determined by a plate count method. Lyophilized powders were rehydrated in 1 mL of PBS and these suspensions were serially ten-fold diluted in MRS broth. All dilutions were dropped into MRS agar and colonies were enumerated after incubation of plates anaerobically at 37°C for 48 h. Results were expressed as % viability of the initial lyophilized powder.

In vitro release studies

Release of the lactobacilli from capsules was carried out to assess the ability of the coating materials to protect the capsules at the physiological pH of the stomach and intestine at 37 ± 0.5°C using a USP dissolution apparatus I rotating at 100 rpm. The coated capsules were tested initially in SGF pH 1.2 containing 0.1 N hydrochloric acid (900 mL). This was followed by replacing the dissolution medium with 900 mL SIF pH 7.4 and the dissolutions were continued for another 3 h. At regular time intervals, the number of viable lactobacilli in the dissolution medium was determined by the normal plate count method.

Statistical analysis

All data were expressed as a mean ± SD. The student’s t test was used for statistical analysis by comparing treatment groups versus control groups. Results were regarded as statistically significant at p < 0.01.

Results and Discussion

Identification of lactobacilli strain

The identification of lactobacilli was initially based on colony morphology, Gram-stain reaction, sugar fermentation profiles and enzyme activities. The isolated strain was identified as L. plantarum by biochemical tests and the API 50 CHL system (bioMérieux, France) with 93.0% confidence. This result was confirmed with rRNA sequencing. rRNA sequences are one of the quickest ways to identify lactic acid bacteria species used in fundamental and applied research [14, 15]. The 16S rRNA gene of the isolate was 100% identical to type strain of L. plantarum ATCC 14917. The strain was assigned as L. plantatrum T23/3 with as accession number of HM051157.

Antimicrobial activity

One important property of a functional probiotics is its anti-pathogenic activity. The antimicrobial ability of L. plantarum T23/3 against five potential gastrointestinal pathogens was assayed. This isolate showed strong inhibitory action on the selected pathogens after an overnight incubation period. L. plantarum T23/3 inhibited both Gram positive (S. aureus 1730) and Gram negative bacteria (E. coli 2601, S. typhimurium 1756, S. sonnei 1549 and V. cholera 3194) with a range of 36-38 mm. The antimicrobial activity of lactic acid bacteria may be due to a number of factors. Generally, the effect of lactobacilli in controlling the proliferation of pathogenic bacteria is by producing a wide range of antibacterial compounds such as organic acids (e.g., lactic acid and acetic acid) and hydrogen peroxide [16]. In fact, the production of lactic acid was enough to inhibit certain bacterial strains. This is because the unionized form of lactic acid triggers a lowering of the internal pH of the cells that causes a collapse in the electrochemical proton gradient in sensitive bacteria, hence having a
bacteriostatic or bactericidal effect [17].

**Antibiotic resistance**

Antibiotic resistance has become a serious problem in treatment of infections caused by a variety of microorganisms due to the indiscriminate use of antibiotics in human medicine. Detection rate of multi-antibiotic resistant bacteria has increased and resistance is common among strains belonging to the gastrointestinal tract of humans. In this study, *L. plantarum* T23/3 was sensitive to ampicillin and chloramphenicol. However, it was resistant to erythromycin, doxycycline, penicillin G, neomycin and tetracycline. One of desirable properties of lactobacilli to be used as probiotic is the resistance to antibiotics. The lactobacilli can effectively protect the natural balance of intestinal microflora during and after therapy by the antibiotics to which they were proved resistant. This strain is also useful in the bio-agents and dairy product manufacture if antibiotics were present in the milk due to antibiotic use in growth promotion and gastrointestinal therapy. The high intrinsic resistance and susceptibility of lactobacillus strains to a range of antibiotics are important since these strains survive during a specific antibiotic treatment. In the treatment of gastrointestinal tract infections, better management is obtained when concurrent therapy is made with probiotic lactobacilli and antibiotics to which they are intrinsically resistant. By doing so, intestinal microflora can recover more quickly [18].

**Survival gastrointestinal transit**

In probiotic selection, this strain needs to clearly demonstrate that acid sensitive strains can colonize and survive in the small intestine [19]. In this study, the effects of different pH values of SGF and SIF on the viability of *L. plantarum* T23/3 were studied (Fig. 1). This isolate remained viable at pH 4.0 for 4 h, but it did not survive at pH 2.0 and 3.0 after 2 h of incubation in SGF.

![Figure 1](http://example.com/figure1.png)

Figure 1  Percent survival of *L. plantarum* T23/3 in simulated gastric fluid at pH 2 (♦), pH 3 (■), pH 4 (▲) (A), simulated intestinal at pH 8 (♦) and bile (oxgall) at 0.3% (w/v) (●) (B) (mean ± SD, n=6)
conditions (Fig. 1A). In the SIF condition, the total number of *L. plantarum* T23/3 showed no loss of viability after incubating at pH 8.0 for 4 h (Fig. 1B). This result indicated that this strain could survive in gastric conditions for up to 2 h so when it moved to the small intestine, at a more neutral pH, it is expected that it will grow, become attached to the epithelium, and eventually start to provide health benefits.

**Aggregation activity**

Cell adhesion is a complex process involving contact between the bacterial cell membrane and interacting surfaces. The ability to adhere to epithelial cells and mucosal surfaces has been suggested to be an important property of many bacterial strains used as probiotics. A variety of surface structures of bacteria are involved in adherence mechanisms including bacterial aggregation between microorganisms of the same strain (auto-aggregation) or between genetically different strains (co-aggregation) and hydrophobic properties is of considerable importance in several ecological niches, especially in the human gut, where probiotics are to be active [20]. In the gastrointestinal tract, aggregation can allow lactobacilli to form a mucosal biofilm and thus prevent the attachment of pathogens [21]. In the

The cell surface hydrophobicity assay was performed to confirm the auto-aggregation characteristic. The property of hydrophobicity is an important mechanism in bacterial adherence. In this study, the results revealed that this strain had a high surface hydrophobicity. In general, lactobacilli strain with that highly negative charged hydrophilic cell surface adhered minimally whereas lactobacilli strains with a slightly negative charged, hydrophobic cell surface adhered strongly [22]. As a salt concentration increased, some of water molecules are attracted by the salt ions, which decreases the number of water molecules available to interact with the charged part of the bacterial cell wall. As a result of increased demand for solvent molecules, the bacterial cell wall-cell wall interactions are stronger than the solvent-bacteria cell wall interactions, the bacterial cell wall then coagulates by forming hydrophobic interactions with each other.

Co-aggregation could be an important factor in maintaining gastrointestinal health because it produces an area around the pathogen where the concentration
of antimicrobial substances produced by the lactobacilli is increased [22]. The result obtained showed that *L. plantarum* T23/3 showed co-aggregation and adhesion with five strains of gastrointestinal pathogens (*E. coli* 2601, *S. typhimurium* 1756, *S. sonnei* 1549, *V. cholera* 3194 and *S. aureus* 1730) as observed with a light microscope (data not shown). Bacterial aggregation between microorganisms of the same strain or between genetically divergent strains is of considerable importance in several ecological niches, especially in the human gut and many authors have reported that the co-aggregation abilities of lactobacilli species might enable it to form a barrier that prevents colonization by pathogenic bacteria [21]. Therefore, the selected strains not only exhibited auto-aggregation and surface hydrophobicity ability, but also caused co-aggregation.

**Adhesion to Caco-2 cell line**

The adhesion of probiotic strains to intestinal epithelial cells is considered as a prerequisite feature for attachment and proliferation in the intestinal environment. The ability to adhere to epithelial cells and mucosal surfaces has been suggested to be an important property of many probiotic bacterial strains [22]. The binding ability of isolates was evaluated using the Caco-2 cells. This cell line has been used as an *in vitro* model for intestinal epithelium and used to screen for adhesive strains [21]. In this study, *L. plantarum* T23/3 was proved to be the most adhesive strain since approximately 90% of added bacteria were bound to Caco-2 cell line (Fig. 3). This result showed that *L. plantarum* T23/3 was able to bind to Caco-2 epithelial cells as did the positive *L. casei* TITR1463 control. Whereas, this strain bound with much higher numbers than that of the negative control of *L. cellobiosus* T13/5 (p < 0.01).

Cell adhesion is a multistep process involving contact of the bacterial cell membrane and interacting surface. In this study, we investigated the competitive binding, exclusion and displacement inhibition of adherence of pathogenic bacteria to Caco-2 cell by adhering *L. plantarum* T23/3. Table 1 represents the inhibition of pathogens adhesion in presence of *L. plantarum* T23/3. All the numbers of adherent pathogens were obviously reduced by co-culture with *L. plantarum* T23/3. The reduction of *E. coli* 2601 and *V. cholera* 3194 adhesion to Caco-2 cells by the lactobacilli was more than 70% when compared to the control (p < 0.01). However, *L. plantarum* T23/3 showed good adhesion and inhibition of pathogen binding and is thereby proving as a suitable probiotic strain.

![Figure 3](image_url)  
**Figure 3** Percent attachment of *L. plantarum* T23/3 on the Caco-2 cells (mean ± SD, n=6)  
(*significant difference from negative control at p < 0.01*)
The freeze-drying process is commonly used for the preservation and storage of microorganisms for industrial applications. The optimal performance of certain strains should guarantee their potential to survive and for stabilization of their metabolic activity [23]. The survival rate of microorganisms varies among the strains and the agents used as the suspending media. In the present studies, lactose was used as a protecting agent to enhance their survival during freeze drying and subsequent storage. In this study, L. plantarum T23/3 strain survived after freeze drying and the data showed no significant differences before and after the process (Fig. 4A). This assured that the lyophilized process did not kill L. plantarum T23/3. This strain remained viable up to 100% in capsules after 12 months of storage at 4°C (Fig. 4B).

Enteric coated capsules are designed to remain intact in a highly acidic environment of the stomach but dissolve rapidly in the neutral environment of the small intestine activity [23]. Eudragit® L-100 was used to coat the hard gelatin capsules for the delivery of L. plantarum

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**Table 1** Percent attachment of E. coli 2601 and V. cholera 3194 to Caco-2 cell line under competition, exclusion and displacement condition with L. plantarum T23/3

<table>
<thead>
<tr>
<th>GI pathogens</th>
<th>Control</th>
<th>Competition</th>
<th>Exclusion</th>
<th>Displacement</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli 2601</td>
<td>100 ± 0.2</td>
<td>74.3 ± 0.5</td>
<td>70.3 ± 0.2</td>
<td>80.3 ± 0.2</td>
</tr>
<tr>
<td>V. cholera 3194</td>
<td>100 ± 1.1</td>
<td>75.9 ± 1.1</td>
<td>73.5 ± 0.3</td>
<td>79.5 ± 0.2</td>
</tr>
</tbody>
</table>

All values are significantly lower than the control at *p* < 0.01

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**Figure 4** Survival of L. plantarum T23/3 before and after freeze drying (A) and storage as a freeze-dried powder into 12% Eudragit® L-100 coated capsule during 12 months at 4°C (B) (mean ± SD, n=6)
T23/3 dry powder form to the intestine. Eudragit® L-100 is an anionic copolymer of methacrylic acid and acrylates which is resistant to the acidic environment present in the stomach but dissolves rapidly in the small intestine [24]. The in vitro release profiles of L. plantarum T23/3 from the freeze dried enteric-coated capsules are shown in Figure 5. The freeze-dried lactobacilli loaded into the Eudragit®L-100 coated capsule did not release the bacterial content in SGF pH 1.2 over 2 h but dissolved, released the L. plantarum T23/3 instantly in the SIF at pH 7.4. The results demonstrated that Eudragit®L-100 coated capsules were successful for intestinal delivery of lactobacilli.

**Conclusion**

The Lactobacillus isolate T23/3 used in this study was identified as L. plantarum and found in vitro to have good probiotic properties. A freeze dried powder of the isolate in capsules survived up to 12 months after storage at 4°C. The capsules were able to protect the release of bacteria in the stomach condition and release completely in the intestine. This strain is well candidates for investigation in vivo studies to illustrate this potential health benefits and this application as novel probiotic strain in the pharmaceutical and food products. However further studies are necessary such as long term stability studies (at least two years), scale up feasibility, safety studies and health benefits in man before product approval or commercialization.

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**Declaration of interest**

The authors report no conflicts of interest.

**References**


Figure 5 Dissolution profiles of L. plantarum T23/3 from the freeze-dried in 12% Eudragit®L-100 coated capsule to delay release of probiotic during 2 h in SGF pH 1.2 and immediately release in SIF pH 7.4 (mean ± SD, n=6)


