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Isolation and Identification of an Indigenous Probiotic Lactobacillus Strain: Its Encapsulation with Natural Branched Polysaccharids to Improve Bacterial Viability

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Abstract

Background and Objective: Probiotics have to reach their site of action in certain numbers in order to exhibit positive health effects. Encapsulation has shown remarkable enhancing effects on probiotic survival in simulated gastric conditions compared to free bacteria. The purpose of this study was identification and evaluation of a potential probiotic strain using encapsulation process by new carriers in order to improve probiotic viability during in vitro simulated conditions.

Material and Methods: A native Lactobacillus was isolated from yogurt, identified as *Lactobacillus casei* PM01 (NCBI registered) and analyzed for probiotic properties alongside established probiotic strains of *Lactobacillus acidophilus* ATCC 43556, and *Lactobacillus rhamnosus* ATCC 7469. Acid and bile resistance, adhesion to Caco-2 cells and antibiotic resistance were evaluated. *Lactobacillus casei* PM01 was encapsulated with alginate, chitosan and natural branched polysaccharides (pectin, tragacanth gum and gum Arabic) by using extrusion technique. Encapsulation efficiency, acidification activity and viability of entrapped *Lactobacillus casei* PM01 in simulated gastric pH were determined.

Results and Conclusion: Based on the results, all the three strains could be considered as potential probiotics, and are good candidates for further in vitro and in vivo evaluation. The results showed that the survival of encapsulated *Lactobacillus casei* PM01 was significantly ($p \leq 0.05$) increased when it was incubated in simulated gastric pH. It can be concluded that indigenous *Lactobacillus casei* PM01 in encapsulated form is introduced as an efficient probiotic strain for using in dairy products.

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1. Introduction

According to FAO/WHO (2002), probiotics are live microorganisms, which (when administered in adequate amounts) confer a health benefit on the host [1]. Microorganisms, before being introduced as probiotics, should have some properties including being generally recognized as safe and non-pathogenic, having acid and bile tolerance, being able to adhere to human intestinal epithelial cells, having antibiotic resistance, and being viable in probiotic production conditions [1,2]. The genus Lactobacillus is one of the most common bacteria used as probiotic. They are gram positive, acid tolerant, catalase negative, and usually rod-shaped [3,4]. It is necessary that the concentration of probiotic micro-organisms in food products be high enough to some specific level. This helps the microorganisms to be present at enough amounts safe, alive, and active in the gastrointestinal tract and play their probiotic role. But studies indicate that, generally, a large number of probiotic bacteria die during production and maintenance processes and harsh condition of the gastrointestinal tract before arriving to their action site. Based on this, one of the most important fields of study is to increase the viability of probiotics. Meanwhile, one of the most efficient and newest methods is the encapsulation of probiotic strains [4]. From microbial point of view, encapsulation is defined

as the covering of a material around the microorganism cells in order to trap and separate them from the environment that results in viability increase at different environmental conditions, and furthermore, targeted release of probiotics in the right time and the right place [5,6]. Calcium alginate is a widely used material in probiotic encapsulation. Alginate has the following advantages: it easily form gel matrices around the bacteria membrane, it does not have any toxic effect on the cell or the body (after consuming the product containing it), it is known as an approved food additive, it is inexpensive, it is easily accessible in laboratory scales, and it is easily dissolved at intestine conditions and releases the alginate-containing cells [7,8]. Many different methods exist for encapsulation of probiotics including spray drying, emulsion and extrusion [5,6,9]. In recent years, different compounds have been used for probiotic bacteria encapsulation namely alginate, starch, chitosan, xanthan gum, gellan, κ-carrageenan, maltodextrin, whey protein, and poly-L-lysine [9]. However, alginate capsules are not resistant to acidic conditions, and their mechanical resistance reduction is proved in the environments containing lactic acid [10]. The presence of compounds like prebiotics in capsule structure, besides offering health-conferring properties, helps to strengthen and improve the capsule's structure [11,12]. Prebiotics are non-digestible or less digestible compounds that intensify the growth and activity of probiotics, and can be specifically used by probiotics [13]. Using natural branched poly-saccharids like pectin, gum arabic, and tragacanth seems to be beneficial as prebiotics. In addition to strengthening the capsule, they possess other advantages including possibility to increase the scale, not using an organic solvent, hard production condition, and helping to increase the growth or the activity of probiotic bacteria for having prebiotic characteristics. Although considerable advances have been made in the field of probiotic encapsulation, still many challenges need to be improved during the proper selection of materials and techniques. Despite that alginate is suitable for the encapsulation, previous studies reported that encapsulation of probiotic bacteria in alginate beads was not able to effectively protect the organisms from high acidity [10,11,14]. In this study, a native strain was isolated from yogurt, and its probiotic properties were investigated. Then new encapsulation carriers as two-layer beads containing alginate and natural polysaccharides (as prebiotic) including tragacanth, pectin, gum Arabic, and chitosan were employed to encapsulate an indigenous *Lactobacillus* (*L.*) *casei* PM01 to protect the bacterial cells in high acidic conditions and increase their viability.

2. Materials and Methods

2.1. Bacterial strains and culture conditions

L. casei (a native Iranian strain isolated from yogurt from Shahre-kord region) was used in this study as a probiotic candidate. *L. acidophilus* ATCC 43556 and *L. rhamnosus* ATCC 7469 were purchased as the standard strains of probiotics [15] from Iranian Research Organization for Science and Technology, and their probiotic properties were compared to the native strain. Lactobacilli were incubated on deMan, Rogosa and Sharpe (MRS) broth at 37º C for 24 h and MRS agar for 48 h.

2.2. Biochemical and molecular characterization of the isolate

Biochemical characterization of the isolate including catalase and oxidase tests, along with production of gas from glucose and carbohydrate utilization tests were performed according to Garcia [16]. In addition to phenotypic characterization methods and biochemical tests, molecular methods were used to identify the isolate [1]. For this purpose, the universal common primers, forward 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and reverse 1492R (5′-GGTTACCTTGTTACGACTT-3′) were used for amplification of 16S rRNA with the PCR technique [17].

2.3. Evaluation of probiotic properties

2.3.1. Tolerance to low pH conditions

The cultures were grown in MRS broth at 37º C overnight, and then centrifuged at 5000×g for 10 minutes. Next the pellets were washed twice in sterile phosphate buffered saline (PBS 0.1 M, pH 7), and resuspended in PBS to reach the initial volume.

MRS (pH 2.5, 3, 4) was prepared and inoculated with overnight bacterial suspensions. After 3 and 4 h incubitions, each sample was cultured on MRS agar plates, and viable bacterial colonies were counted [18].

2.3.2. Bile tolerance test

MRS broths supplemented with different concentrations of bile salts (0.3 and 0.7%) were prepared and inoculated with overnight bacterial suspensions. Then they were incubated at 37º C for 8 h. Absorbance at 600 nm was measured by using spectrophotometer at 0 to 8 h, and a coefficient of inhibition was calculated as follows [19]:

$$C_{inh} = \frac{(T_8 - T_0)_{control} - (T_8 - T_0)_{treatment}}{(T_8 - T_0)_{control}} \quad \text{Eq. 1}$$

where, (T_8-T_0) represents the differences in absorbance between T_0 (zero hours reading) and T_8 (reading on the 8th hour) for control or treatment. C_{inh} for 2 and 4 h incubations in the presence of oxgall bile were also measured. Absorbance of broth in the absence of bile salt at different times was considered as control sample.

2.3.3. Adhesion assay

Adhesion of the probitic cultures was measured as per the method described by Jacobsen et al. [16]. The cell suspension with 1×10^8 cells was prepared in 4 ml complete DMEM medium was transferred to each well of six-well tissue culture plates. The medium was changed every alternate day. When cells reached 80 per cent confluency, the medium was replenished each day consecutively for 15 days for both the cell lines. Adhesion assay was done 24 h after removing spent medium and cells were fed with DMEM medium without antibiotics. The cells were then washed twice with 3 ml phosphate-buffered saline (PBS, pH 7.4). An aliquot of two ml of DMEM (without serum and antibiotics) was added to each well and incubated at 37º C for 30 min. Different probiotics cultures (at 1×10^8 CFU) suspended in 1 ml DMEM medium (without serum and antibiotics) were added to different wells. The plates were incubated at 37° C in 5% CO_2 , 95% air for 2 h. The monolayers were washed five times with sterile PBS (pH 7.4). The adhesion score was measured by enumerating adhered bacteria per 20 different microscopic fields.

Methanol was added to each well of six-well plates at the rate of 3 ml followed by incubation for 10 minutes at room temperature. Methanol was completely removed, and the fixed cells were stained with Gram stain for 20 minutes at room temperature. The plates were air dried and examined under oil immersion microscope. The number of bacteria was counted in 20 random microscopic fields and were grouped into non-adhesive (≤40 bacteria), adhesive (41-100 bacteria) and strongly adhesive (>100 bacteria).

2.3.4. Antibiotic resistance

Antibiotic susceptibility was determined by disc diffusion method according to the protocol suggested by the producer (Padtan Teb Co., Iran). The antibiotics tested included: Ampicillin 10 µg, Cephalothin 30 µg, Vancomycin 30 µg, Tetracycline 30 µg, Chloramphenicol 30 µg, Erythromycin 15 µg, Clindamycin 2 µg, Doxycycline 30 µg, Penicillin 10 µg, Cefazolin 30 µg, Oxacillin 1 µg, Cephalexin 30 µg, Nalidixic acid 30 µg, Gentamicin 10 µg and Enrofloxacin 5 µg. The above antibiotic concentrations are per disc.

2.4. Encapsulation of newly probiotic strain

2.4.1. Encapsulation procedure

The encapsulation procedure employed in the present study used a slightly modified version of the extrusion technique previously described by Trabelsi et al. [21]. Three types of alginate capsule of branched polysaccharides were prepared by ionotropic gelation, with the following composition: alginate pectin-chitosan (APC), alginate-gumarabic-chitosan (AGC), and alginate-tragacanth-chitosan (ATC). All glass ware and solutions used in the protocols were sterilized at 121º C for 15 min. Sodium alginate (2% w v^{-1}), branched polysaccharides (gum Arabic, pectin and tragacanth) (0.5% w v^{-1}), glycerol (5% v v^{-1}) and tween 80 (0.02%) solution was prepared in distilled water and stirred for polymer hydration. After sterilized at 121º C for 20 min, the fresh cellular concentrate (10^{11} CFU ml^{-1}) was suspended in the alginate-gum mixture under stirring. The peristaltic pump and sterile syringe (31 G) were used to bring 5 ml of the alginate-gum mixture drop wise into 25 ml of the gelling solution. Sterilized calcium chloride solution (0.45 M, pH 7) was used as gelling solutions. The droplets immediately formed gel spheres. To obtain complete gelation, the beads were stirred for 30 min. The beads were washed three times with distilled water and then transferred to 25 ml of 0.8% w v^{-1} chitosan solution in 0.1% v v^{-1} acetic acid solution. The beads were stirred gently with a magnetic bar for 15 min to evenly coat the surface of the alginate beads.

2.4.2. The encapsulation yield

The encapsulation yield (EY), which is a combined measurement of efficacy of entrapment and survival of viable cells during encapsulation, was calculated as follows:

$$EY = (N/N_0) \times 100 \qquad \text{Eq.2}$$

where, N is the number of viable encapsulated cells released from the capsules and N_0 is the number of free cells added to the polymer mixture prior to extrusion [22]. Sodium citrate solution (0.1 M) was used to release the cells from the capsules [23].

2.4.3. Acidification activity

Free and encapsulated cells of *L. casei* PM01 were inoculated in 10 ml MRS broth to determine their acidification activity. Then they were incubated at 37º C for 48 h, and the rate of acidification was established by recording the pH for each culture at different intervals over a period of 48 h [11].

2.4.4. The viability of bacteria in simulated gastric pH 2 and 2.5

The viability of bacteria in simulated gastric pH was measured according to method of Trabelsi et al. with slight modifications [21]. The encapsulated and free bacteria were added to 10 ml MRS broth, previously adjusted to pH 2 and 2.5. The samples were then incubated for 3 h at 37º C in shaker incubator. Then they were centrifuged at 5000× g for 10 min; the sediment washed with peptone water, and sodium citrate solution (0.1 M) was used to release the bacteria from the capsules. Serial dilutions were made and plated on MRS agar. The plates were then incubated at 37º C for 48 h.

2.5. Statistical analysis

The results were assessed using the least square difference (LSD) and analysis of variance (ANOVA) at

$p \leq 0.05$ level of significance using the SPSS software (ver. 16.0). Graphs were plotted by Excel 2013. All experiments were done in triplicate.

3. Results and Discussion

3.1. Identification of native isolate

The results of the biochemical tests for the isolate showed that it was rod-shaped, gram-positive, catalase-negative, oxidase negative, lacking the ability to produce gas from the fermentation of glucose, and able to consume lactose, mannitol, sucrose and sorbitol sugars. The results were corresponded to the Berge's manual of determinative bacteriology.

The results of the sequencing of the PCR product were analyzed in the NCBI database and high similarity of the genome (99% homology) with *L. casei* PM01 was confirmed. The sequence was then submitted to the EMBL database by HG931728 accession number.

3.2. Probiotic properties

3.2.1. Acid tolerance

According to the Table1, the effect of acidic conditions (pH 2.5, 3.0, 4.0) on the viability of *L. acidophilus, L. rhamnosus,* and *L. casei* PM01 strains showed that after 3 and 4 h of incubation period, the number of bacteria were not less than 10^6 CFU ml^{-1}.

It has been reported that low pH environments reduce the probiotics viability due to inhibition of the metabolic activities and growth of *L. acidophilus* [11,24]. The number of probiotics severely decreased when exposed to the simulated stomach juice at pH 1.5 for 3 h incubation [7]. Jacobsen et al. by investigating the viability of 44 lactobacillus isolates, demonstrated that the pH value of 2.5 causes the maximum damage to the strains in such a manner that only 29 strains survived for 4 h and none of them showed any growth. They also reported a large difference in the viability of *L. acidophilus* and bifidobacterium isolates at pH values between 2 and 3 [20].

3.2.2. Bile tolerance

Probiotic bacteria should be able to grow in 0.15-0.30% of bile salts [25]. So, the ability of *L. casei* PM01 to grow in the presence of 0.3% and 0.7% oxgall bile was examined. Based on the calculated coefficient of inhibition ($C_{inh} \leq 0.4$), *L. casei* PM01 had good tolerance to the tested bile salt concentrations (Table 2).

Bile salts as cholesterol polar derivatives are very effective detergents. Since they have both polar and non-polar regions, upon exposure of bacteria to bile acids, cellular homeostasis disruptions occur. Dissociation of lipid bilayer and integral protein of their cell membranes results in the leakage of bacterial content and ultimately cell death [26]. Resistance to bile salts of some strains is related to the activity of bile salt hydrolase in which the hydrolysis of bile salts results in their toxicity and side effects reduction [27].

Table 1. Survival of Lactobacillus strains at pH 2.5, 3.0 and 4.0 after 3 and 4 h incubation periods

strain	pH 2.5		pH 3		pH 4	
	3 h	4 h	3 h	4 h	3 h	4 h
L. acidophilus	6.23±0.09	6.26±0.16	6.28±0.13	6.34±0.29	6.32±0.15	6.49±0.52
L. rhamnosus	6.20±0.33	6.26±0.19	6.36±0.07	6.59±0.19	6.96±0.26	7.38±0.08
L. casei PM01	6.40±0.13	6.45±0.23	6.49±0.07	6.54±0.26	6.70±0.17	6.97±0.05

Each value in the table is the mean value of log CFU ml^{-1} ± SD of three trials.

Table 2. Coefficient of inhibition in MRS broth supplemented with 0.3% and 0.7% oxgall after 2, 4 and 8 h incubation periods

Ox-bile (w v^{-1})	Time (h)	*L. acidophillus*	*L. casei*	*L. rhamnosus*
0.3%	2	1.60	0.93	1.03
	4	0.34	0.59	0.36
	8	0.35	0.33	0.36
0.7%	2	2.60	0.91	0.22
	4	0.65	0.70	0.50
	8	0.43	0.42	0.25

3.2.3. Adhesion to Caco-2 cells

After the cell adherence test and counting the bacteria, it was revealed that Lactobacillus strains were attached to Caco-2 culture cells with different gained scores in 20 microscopic fields as 198, 159, and 63 for *L. acidophilus*, *L. rhamnosus*, and *L. casei* PM01 strains, respectively (Figure 1). The results showed that all three strains are adhesive; however, according to their gained scores, *L. casei* PM01 is less adhesive than two other strains, which had more bacteria seen on the cells after five times washing.

Matijasic et al. investigated the attachment of two strains of *L. gasseri* to Caco-2 cells. They showed that the ability to attach this cell line is different [28]. Chauviere et al. also investigated the attaching capability of 25 strains of *L. acidophilus* and demonstrated that the attachment capability is variable even in one species of Lactobacilli and depends on the strain [29]. As it was specified by Kleeman et al. in 1982, bi- or trivalent cations can play the ionic bridge role between the bacteria's surface and the epithelial cells, and be crucial in most adherence systems. These researchers showed that Lactobacillus adherence to the intestinal cells is enhanced by bivalent calcium cations [30]. It seems that the adherence of Lactobacillus strains to Caco-2 cells is mediated by cell surface components and extra-cellular factors [31].

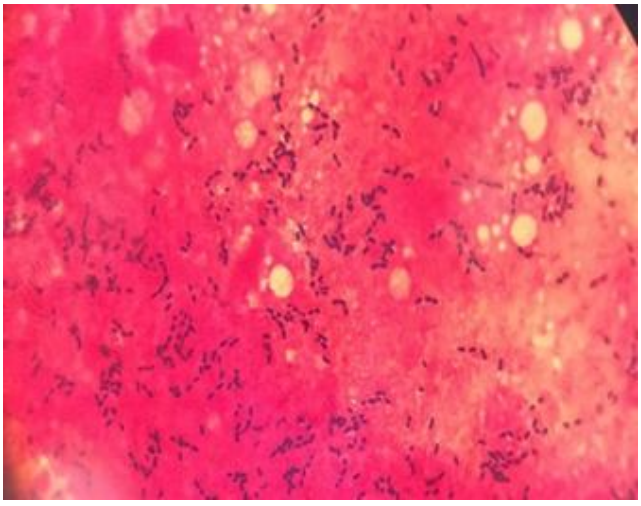

Figure 1. Adhesion of Lactobacillus strains to Caco-2 cell line examined by light microscopy after Gram staining.

3.2.4. Resistance to antibiotics

Antibiotic resistance in bacteria can be inherent or acquisitive. The inherent resistance occurs naturally and is a part of species properties, while acquisitive resistance has roots in mutation or acquiring external DNA from other bacteria [32]. However, studying on the genes related to the resistance is necessary for establishing the safety of probiotic strains.

Based on the derived results, resistance to antibiotics' pattern expresses that all three strains are resistant to Vancomycin, Nalidixic acid, Getamicin, and Enrofloxacin antibiotics. On the contrary, they are all susceptible to Ampicillin, Clindamycin, Tetracycline, Doxycycline, Cephalothin, Chloramphenicol, Penicillin, Cefazolin, Erythromycin, Oxacillin, and Cephalexin antibiotics (data not shown). But it is to be noted that the inhibition zone diameter, in cases of susceptibility to specific antibiotics, is different from one strain to another. So, more complementary tests are needed in order to determine the minimum inhibitory concentration for each antibiotic and strain.

3.3. Encapsulation of *L. casei* PM01

Encapsulation was done by the extrusion method, and finally, alginate capsules and branched polysaccharides with chitosan covering containing *L. casei* PM01 were formed.

As shown in Figure 2, capsules have similar size. Despite using the smallest needle diameter of the syringe, the capsule diameters ranged between 1 and 1.5 mm. Martin-Dejardin et al. encapsulated probiotic bacteria in 1-2 mm diameter by the extrusion method [23].

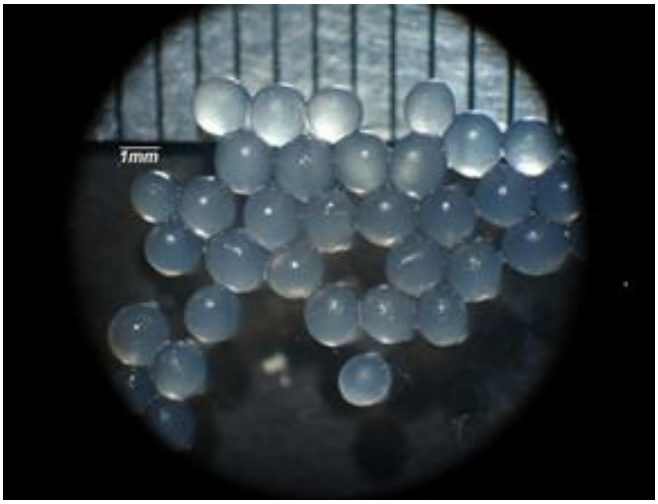

Figure 2. Capsules containing *L. casei* PM01 under light microscope.

Increasing the size of capsules increases their protective effect against unfavorable environmental conditions [33]. It is reported that capsules under 100 µm cannot enhance the viability of probiotics in acidic conditions of the stomach to a significant level; on the other side, capsules larger than 1mm cause non-uniformity and granulation of food matter tissue [14].

3.3.1. Encapsulation efficiency

The results of the encapsulation efficiency of *L. casei* PM01 strain (Figure 3) showed that the encapsulation process to form ATC capsules has 85.1% efficiency whereas this percentage for APC and AAC capsules is 72.7% and 84.2%, respectively. The lower efficiency of APC is perhaps due to the higher viscosity of the encapsulation solution. So, the bacterial cells may be lost

because of attachment to the internal surfaces of hose, syringe and container.

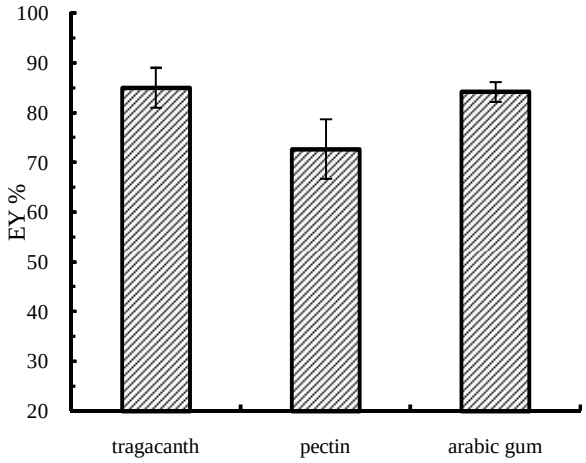

Figure 3. The encapsulation efficiency of *L. casei* PM01 entrapped in different beads.

It has been reported that the encapsulation efficiency for *L. casei* by the extrusion method varies from 54.3% to 79.2% depending on total polymer concentration in the cell suspension [12]. The encapsulation efficiency depends upon different factors like encapsulation materials as well as on their concentrations, process techniques and methods for cell counting. For example, trisodium citrate reduces the efficiency because of its toxic effect on the viability of bacteria [34]. Although we tried to shorten the exposure time of capsules with trisodium citrate by homogenization, probably the bacteria released earlier are more exposed to. Reid et al. pointed out some bacterial cells lysed in $CaCl_2$ solution and encapsulation efficiency decreased [35]. However, the results showed that the encapsulation efficiencies of three prepared capsules were ca. 80%. It may be due to chitosan covering (as a multi-cation composition) around the negatively charged alginate capsules that creates covered capsules and results in more physical and chemical stability of the capsules along with reducing the destructive effect of anti-gel and calcium ion involving factors in the structure.

3.3.2. Acidification activity

Figure 4 shows the trend of pH variations after adding free and encapsulated bacteria to the MRS broth culture for 48 h. The reduction of MRS broth pH for encapsulated L. casei PM01 is less than that of free cells in the same time duration. In other words, the longer time is required to decrease culture medium pH by encapsulated bacteria than that of free cells. It seems that capsules covering the cells reduce the transport velocity and the metabolic activities of probiotics.

In a similar study, Sultana et al. reported the pH reduction time from 6 to 5 for encapsulated *L. acidophilus* and *L. casei* PM01 with calcium alginate about 30 h [11].

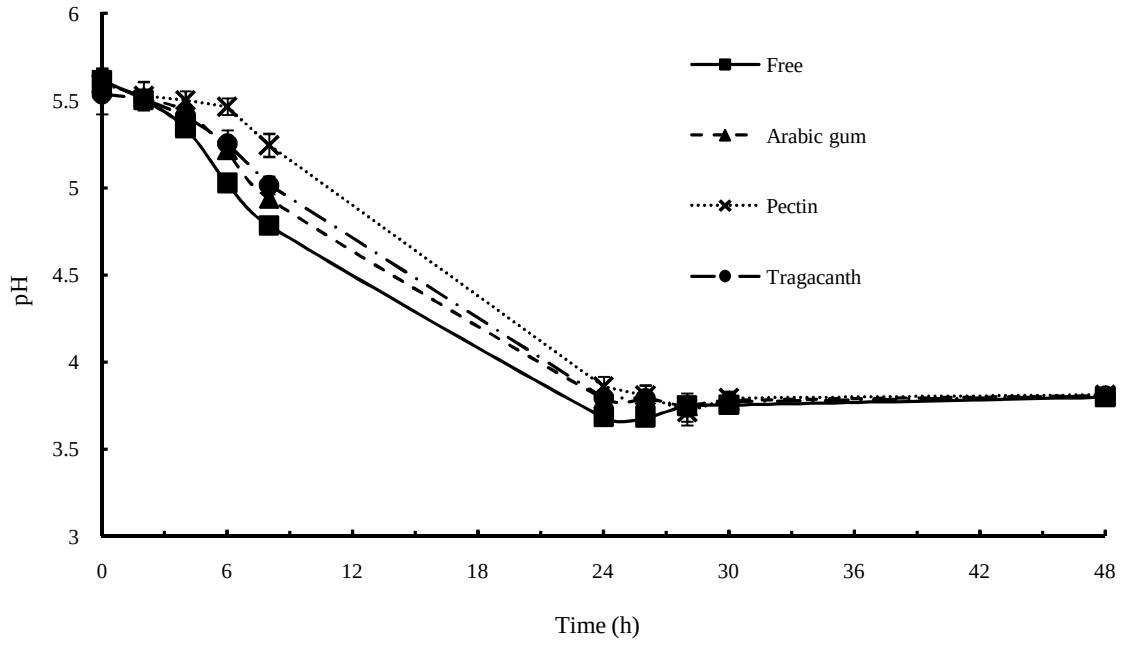

Figure 4. Trends of pH reduction during the acidification process by the encapsulated and free cells of *L. casei* PM01.

Homayouni et al. also found that the required time to decrease the pH of MRS broth to 4 was about 50 h for encapsulated lactobacillus [36]. pH reduction in the present study is similar to the above mentioned studies but in our experiments, as it can be seen in Figure. 4, the difference between the pH reduction of encapsulated bacteria in comparison to the free cells is less than in the previous reports. The averages of the pH magnitude of free cells compared to pectin-encapsulated cells in acidification process were significantly reduced (LSD method, $p \leq 0.05$). The trend of acidification activity of encapsulated bacteria in tragacanth and gum Arabic was the same. Larisch et al. also understood that sodium alginate and poly lysine cover increase the pH reduction time compared to free cells by 17%. They reported that if sufficient concentration of cells is loaded into the capsules, the capsules' wall and matrix would have no significant effect on lactic acid production and release [37].

3.3.3. Acid resistance

The viability of *L. casei* PM01 was investigated in three different encapsulation conditions for 3 h at pH 2 and 2.5 (similar to gastric pH). As illustrated in Figure. 5, the encapsulation process has improved the viability of *L. casei* PM01 in low pH conditions significantly. The results of ANOVA showed that a remarkable difference exists between the responses ($p \leq 0.05$). By comparing the averages using LSD method, it is concluded that there is no significant difference between the three branched polysaccharides in the viability of *L. casei* PM01 at pH 2 and 2.5 though all the three types enhanced the viability of the cells as compared to the free cells.

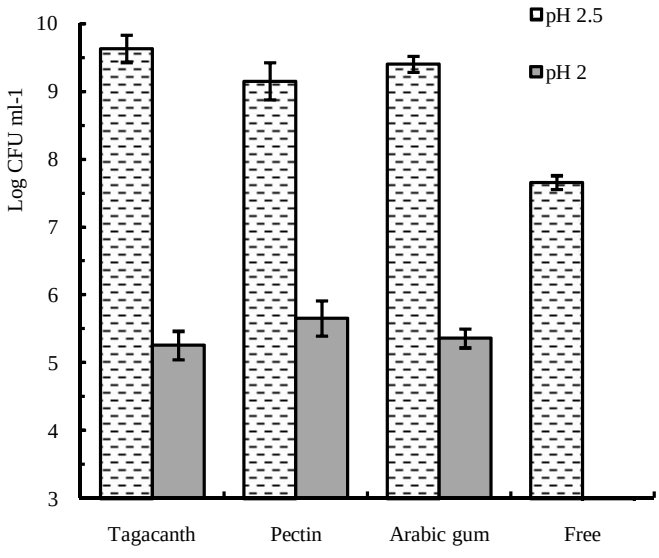

Figure 5. Survival of encapsulated *L. casei* PM01 over 3 h of exposure to low pH media (pH 2 and 2.5).

It has been reported that encapsulation in alginate beads had no significant effect on the viability of probiotic cells in stomach acidic conditions [11]. On the contrary, Mandal et al. reported that the encapsulation (with calcium alginate) increased the viability of *L. casei* NCDC-298 at pH 1.5 [7]. Chandramouli et al. suggested that probiotics encapsulation (in calcium alginate) under optimum conditions improves their viability in simulated gastric pH compared to free bacteria [38]. Ayub and Brinques enhanced the viability of *L. plantarum* by calcium alginate and chitosan encapsulation in similar stomach juice conditions [39].

4. Conclusion

In this research, a native Lactobacillus strain (From Shahre-kord region) was isolated from yogurt and identified as *L. casei* PM01 by morphologic, biochemical and molecular biology tests. The main experiments were conducted and confirmed that this is a probiotic bacterium. However, it is suggested to perform complementary tests such as hemolytic activity, resistance to gastric juice (pepsin and trypsin), transferring antibiotic resistance genes, antimicrobial activity, ability to reduce pathogen adhesion to surfaces, cholesterol removal of blood serum, and bile salt hydrolase activity.

In an attempt to increase the viability of probiotic bacteria, encapsulation method was used as a solution to protect the bacteria from unfavorable conditions. Alginate as a natural polymer was chosen as the main material forming capsules. Since alginates are instable in phosphate environments and cells are released undesirably from the capsules, branched polysaccharides like pectin, tragacanth, and gum Arabic were used to improve the capsules strength and increase the activity of probiotic bacteria as prebiotics. Meanwhile, the negatively-charged alginate capsules were covered by a positively-charged chitosan layer. The results showed that the viability of the encapsulated bacteria at pH 2 and 2.5 was significantly increased in comparison to the free cells ($p \leq 0.05$). It was also revealed that the trapped bacteria within the capsule, when placed in a suitable environment (MRS), are viable and active and capable of doing acidification by lowering the pH in their environment.

It can be concluded that the encapsulated *L. casei* PM01 has a great potential to be used in functional foods as a combination of probiotics and prebiotics.

5. Acknowledgements

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6. Conflict of Interest

The authors declare that there is no conflict of interest.

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جداسازی و شناسایی یک سویه لاکتوباسیلوس پروبیوتیک بومی: پوشینه‌دارسازی سویه با پلی‌ساکاریدهای شاخه‌دار طبیعی با هدف بهبود زنده‌مانی باکتری

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چکیده

سابقه و هدف: باکتری‌های پروبیوتیک برای داشتن اثرات سودمند سلامتی‌بخش، باید به تعداد مشخص به محل اثرشان برسند. پوشینه‌دارسازی به‌طور قابل ملاحظه‌ای، زنده‌مانی پروبیوتیک‌ها در شرایط شبیه‌سازی شده معده را در مقایسه با سلول‌های آزاد افزایش می‌دهد. هدف از این مطالعه شناسایی و ارزیابی سویه‌ای با پتانسیل پروبیوتیکی، با استفاده از فرایند پوشینه- سازی به‌وسیله حامل‌های جدید است تا قابلیت زنده‌مانی در شرایط شبیه‌سازی شده معده بهبود یابد.

مواد و روش‌ها: سویه بومی لاکتوباسیلوس از ماست جداسازی و پس از انجام آزمون‌های زیست شیمی و مولکولی، به عنوان *لاکتوباسیلوس کازئی* PM01 شناسایی و در پایگاه داده‌های NCBI ثبت شد و ویژگی‌های پروبیوتیکی آن در کنار اثبات پروبیوتیک بودن سویه های *لاکتوباسیلوس اسیدوفیلوس* ATCC 43556 و *لاکتوباسیلوس رامنوسوس* ATCC 746 مورد بررسی قرار گرفت. مقاومت به اسید و نمک‌های صفراوی، چسبندگی به سلول های Caco-2 و مقاومت آنتی بیوتیکی مورد ارزیابی قرار گرفت. *لاکتوباسیلوس کازئی* PM01 به روش اکستروژن با آلژینات، کیتوزان و پلی ساکاریدهای شاخه‌دار طبیعی (پکتین، صمغ عربی و کتیرا) پوشینه‌دار شد. بازده پوشینه‌دارسازی، فعالیت اسیدزایی و زنده‌مانی *لاکتوباسیلوس کازئی* PM01 در pH شبیه‌سازی شده معده تعیین گردید.

یافته‌ها و نتیجه‌گیری: براساس نتایج به دست آمده، هر سه سویه می‌توانند به عنوان پروبیوتیک‌های بالقوه در نظر گرفته شوند و انتخاب‌های مناسبی برای ارزیابی‌های بعدی برون تن و درون تن می باشند. نتایج نشان داد زنده‌مانی سلول‌های *لاکتوباسیلوس کازئی* PM01 ریزپوشینه‌دارشده هنگام گرمخانه‌گذاری در شرایط شبیه‌سازی شده pH در مقایسه با سلول‌های آزاد به طور معنی‌داری افزایش یافت ($p\leq0.05$). پس سویه بومی *لاکتوباسیلوس کازئی* PM01 به شکل ریز پوشینه‌دار شده می‌تواند به عنوان یک سویه پروبیوتیک کارآمد برای استفاده در فرآورده‌های شیر معرفی شود.

تعارض منافع: نویسندگان اعلام می‌کنند که هیچ تعارض منافعی وجود ندارد.

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