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INVESTIGATION OF LIPASE INHIBITORY ACTIVITY OF NOVEL PROBIOTICS ISOLATED FROM IRANIAN DAIRY PRODUCTS

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ABSTRACT

The primary cause of metabolic syndrome is obesity, a state in which the body accumulates stockpiles of fat. Lessening of assimilation of dietary fats via suppression of lipase enzyme of the pancreas may incite weight loss. In this work, 21 isolates of lactic acid bacteria (LAB) were monitored for potential novel probiotic traits by means of in vitro methods, comprising of bile salt and acid tolerance, adhesion and lipase inhibitory activity. The findings illustrated that *L. fermentum* sp D9, *L. fermentum* sp S14 and *L. fermentum* sp S8, *L. paracasei* S11 and *L. brevis* S15 demonstrated prominent potential probiotic features as well as lipase inhibitory activity *in vitro*. The traits of opted isolates were as good as to those of *L. rhamnosus* PTCC 1637, a similar strain to *L. rhamnosus* GG, which possessed an antidiabetic aptitude. These findings offered that the abovementioned isolates could be administrated as potent anti-obesity probiotics. It should be noted that, more investigations are necessary to evaluate the inhibitory activity of lipase from these strains.

Keywords: Lipase, Inhibitory Activity, Lactic Acid Bacteria, HT-29 Cell Line, Probiotics

INTRODUCTION

One of the chief contributing factors to the metabolic syndrome is obesity and overweight, a set of interconnected metabolic circumstances that impressively escalate the hazard of emerging coronary heart disease illness (**Ashoori *et al.*, 2020; Jung *et al.*, 2014; Sadraeian *et al.*, 2022b**). These circumstances comprise of resistance to insulin (and type 2 diabetes mellitus), hypertension, non-alcoholic and dyslipidaemia steatohepatitis (**Azarang *et al.*, 2020; Chen *et al.*, 2014; Martin *et al.*, 2015a, 2015b**) which are strictly associated with an imbalance among energy consumption and spending (**J. S. Lee *et al.*, 2021**). Recently, obesity has been categorized as an illness instead of a symptom (**McGee *et al.*, 2005; Sadraeian *et al.*, 2022a**). Globally, over 310 million individuals are regarded obese, and the present corpulence ratio in Iran has elevated from 30.2% in 2007 to 36.7% in 2016 for adults 18 years of age and older (**Moghimi-Dehkordi *et al.*, 2020**).

The most significant pathogenic aspect of obesity is the overgrowth of adipose tissue. It is generally believed that overgrowth is caused by an augmented number of adipocytes (hypertrophy) as well as by the growth of new adipocytes (hyperplasia) via stromal preadipocyte differentiation (adipogenesis) (**McGee *et al.*, 2005**). Adipocytes are accountable for maintaining an appropriate level of energy, for instance, the storage of calories in the form of lipids, the mobilization of energy resources upon hormonal elicitation, and modulating body operations via secretion. It has been demonstrated in recent studies that adipose tissue plays a more significant role than previously believed in regulating animal physiology and metabolism, as well as that most obesity-associated pathogenic events can be attributed to the activity of adipocytes. As a result, modifying hypertrophy and hyperplasia to reduce adipose tissue overgrowth could be a useful method of treating obesity (**McGee *et al.*, 2005**).

Most of the drugs that are administrated for obesity treatment possess side effects like hypertension, stomachache, constipation, anxiety, headaches, and insomnia (**Gholami *et al.*, 2020; D.-J. Kim *et al.*, 2011; Y. Lee *et al.*, 2008**). In this regard, the replacement for medicines are functional foods that attracts much of interest (**S.-J. Lee *et al.*, 2014**). The development of probiotics as a functional food has opened new era in medicine and health being. They are alive microorganisms that deploy several advantageous impacts on the host (**de Melo Pereira *et al.*, 2018; Wulandari *et al.*, 2020**). The typical heterogeneous community of lactic acid bacteria (LAB) consists of genus *Lactobacillus*, which are commonly utilized as probiotics (**Kariyawasam *et al.*, 2020; Montazeri-Najafabady *et al.*, 2021**). In this context, various investigations have confirmed the role of LAB in regulation of gene expression, including in energy homeostasis to subside obesity (**Miyazawa *et al.*, 2012; Ommati *et al.*, 2022**).

Dietary supplements have become increasingly popular in recent years as a means of controlling weight gain without dieting, thereby encouraging the development of healthy food products and their production (**Kearney, 2010**). One of the most prominent probiotic products available on the worldwide market is fermented milk because of the buffering properties that allow bacteria to grow and survive (**Mazloom *et al.*, 2019**). It is believed that most probiotic bacteria nowadays belong to the genera *Lactobacillus* and *Bifidobacterium* (**Marissen *et al.*, 2019**), but other genera, such as *Lactococcus*, *Enterococcus* and *Propionibacterium*, are also regarded as probiotic bacteria (**Mazloom *et al.*, 2019**). Furthermore, *Lactobacillus delbrueckii* subsp. *bulgaricus* (LDB) along with *Streptococcus thermophilus* have been proposed as prospective probiotics, and *Lactobacillus* has been utilized in order to foster human healthiness in fermented foods since ancient times (**Duc *et al.*, 2004**).

The digestion of dietary lipid endures various multifaceted processes before mucosal absorption in the bowel. The crucial enzyme for lipid assimilation is pancreatic lipase due to the most of lipid hydrolysis by pancreatic lipase that is taken placed in the duodenum, which breaks down dietary fats (dietary triacylglycerol) to fatty acids and glycerol (**Golkar *et al.*, 2021; Kumar *et al.*, 2021**). Dietary triacylglycerol, the major source of dietary fat, is not directly absorbed in the intestine unless it is hydrolyzed by pancreatic lipase. The pancreatic lipase serves as a catalyst in the hydrolysis and digestion of fat, cholesterol esters, and fat-soluble vitamins by transporting them to the duodenum after being excreted by the pancreas. A significant source of dietary fat, triacylglycerol, is not directly absorbed in the intestine unless it has been hydrolyzed by pancreatic lipase. Thus, inhibition of lipase-mediated fat digestion is a highly effective strategy to reduce dietary intake of triacylglycerol (**Irajie *et al.*, 2016; Tucci *et al.*, 2010**).

One of the strategies to avoid the administration of dietary supplements or drugs is the consumption of foods that are natural or functional (including fermented dairy products) to decrease lipid intake (**Buchholz *et al.*, 2016; Ishimwe *et al.*, 2015; Marrelli *et al.*, 2014**). The anti-obesogenic influence of numerous palatable fruits and herbal extracts has been demonstrated over the past few years, and the key mods of action are recognized as an escalation in spending of energy, repression of appetite, inhibition of lipase and regulating of lipid metabolic process and differentiation of adipocyte (**Gholami *et al.*, 2022; Sun *et al.*, 2016**). The role of probiotics and fermented dairy products in inhibiting and decreasing obesity has also been extensively inspected in human and animal models, and several modes of action have been suggested (**Barengolts, 2016; Mazloom *et al.*, 2019; Sadraeian *et al.*, 2013**). However, as far as we know, there are a few studies about LAB lipase suppressor in native dairy products. In this study, we evaluated the prospect of ant-lipase activity effects of 13 isolates of lactic acid bacteria (LAB)

from native Iranian dairy products by studying the inhibiting pancreatic lipase *in vitro* that could be helpful for curing obesity via inhibitory activity against lipase enzyme.

MATERIAL AND METHODS

Isolation of *Lactobacillus* strains

A total of 40 samples of traditional dairy products, including milk, yoghurt and dough (Iranian fermented milk product) were collected from 23 different place of Fars province, Iran. One gram of every dairy specimen was dispersed in 2% w/v peptone water and vortexed for 30 seconds. One mL of each sample was transferred to ten mL of MRS broth media (Merck, Germany) in a microaerobic environment at 5% CO_2. These dilutions (0.01 mL) were poured on MRS agar plates (Merck, Germany) followed by incubation at 48 hours. After choosing the colonies on the growth agar plate, they were moved to a broth culture medium followed by incubation at 37°C for 24 hours. For further analysis, the isolates were maintained with 10% skim milk (w/v) and 30% glycerol (w/v) at 70 °C (**Mohkam, Nezafat, *et al.*, 2016**).

Pancreatic lipase inhibitory activity

The method of **Lee *et al.*** (1993) for lipase activity assay was applied with a few modifications (**Y. P. Lee *et al.*, 1993**). A porcine pancreatic lipase was used to measure pancreatic lipase activity (Sigma). 0.1 mg/mL of a solution of the sample was solubilized in water and 0.061 M (pH 8.5) Tris-HCl buffer and 0.167 mM p-Nitrophenylpalmitate (PNP) buffer were added together in the chamber of a plate, and 0.3 mg/mL of the lipase sample was then introduced to initiate the enzymatic reaction. The absorbance of the solution was calculated by means of a microplate reader following incubation at 25 °C for 10 minutes.

Identification of *Lactobacillus strains*

The isolate identification was initially established by examining their morphological, cultural, and biochemical traits using the microtechnique method developed by Gusils et, al (**Gusils *et al.*, 2004**). Then after,16s rRNA analysis was performed in order to confirmation of isolates using the universal 16s rRNA primers (RW01 5' -AACTGGAGGAAGGTGGGGAT-3' DG74 5' AGGAGGTGATCCAACCGCA-3') (**Mohkam, Rasoul-Amini, *et al.*, 2016**). Preliminary denaturation was performed for 20 min at 95°C. Then after, 35 cycles denaturation was done for 1 min at 94°C, annealing at 58°C for 50 sec and extension at 72°C for 1 min. The ultimate extension was done for 1 min at 72°C. PCR amplicons were purified and then sequenced at Cinnagen, Iran. Sequences were aligned with formerly available bacterial 16S rRNA sequences by means of Blast in the databases of NCBI reference.

Acid and bile salt tolerance

The endurance of LAB isolates to acid and bile salts was evaluated using the method described by Duc et al. 2004, with some minor modifications (**Duc *et al.*, 2004**). Briefly, MRS broth with a pH of 2.5 was created and seeded with bacterial suspensions of all the bacterial isolates. Samples of 0.5 mL were investigated at 0 and 3 hours to determine cell viability and total cell count. Enumeration on MRS agar plates was performed at 37 °C for 24 hours at pH 6.

For the purpose of assaying bile salt endurance, MRS broth containing Oxgall 0.2-1% was prepared and inoculated with 1 ml of 48-hold culture. Aliquots were taken at 1, and 3-hour intervals and then cultured onto MRS agar plates. After incubating these plates at 37 °C for 24 hours, colonies were qualitatively determined (+ for the lowest colony observation and 4+ for the highest colony observation).

Adhesion studies of LAB isolates

Strains were cultivated in MRS broth (Himedia, India) at 37 °C for 18-24 h and rinsed twice with PBS. A suspension of bacteria was then prepared up to $10^7 - 10^8$ CFU ml-1 in RPMI 1640 cell culture media enriched with HEPES (as determined by optical density reading to estimate CFU). The adhesion to HT29 cell line [an epithelial cell line that secretes mucus] was investigated in accordance with the procedures provided by **Rowan *et al*.** (2001) with some minor amendments (**Rowan *et al.*, 2001**). In brief, cells were inoculated in 24-well cell culture plates (105 cells per well). The monolayers of cells were cultivated for at least 20 days in 5% CO_2 at 37°C, by utilizing an all-inclusive minimum standard medium (RPMI1640) comprising 10% fetal calf serum. Prior to this, HT-29 cell line was purchase from Pasteur Institute cell bank (Tehran branch, Iran). The cells were cultivated in RPMI 1640 cell culture media enriched with 10% in activated fetal calf serum following by incubation in 5% CO_2 for 2 h at 37°C. after reaching to 70% confluency, the cells were scratched from flasks and subcultured again if needed. For maintain long-term preservation, 1% DMSO was added to each tube containing appropriate number and stored them at liquid nitrogen.

Following the preparation of the monolayers for the assays, the cell monolayers were rinsed a single time with a complete culture medium and subsequently seeded with 1 ml of 10^7–10^8 CFU bacteria in each well (by preparing a bacterium to cell fraction of 100: 1) using a complete culture medium containing HEPES (0.01 mol l^{-1}) in three replicates, followed by a period of incubation in 5% CO_2 for 2 h at 37°C. The control chambers (not containing cell lines) were filled with the corresponding quantity of bacteria and kept incubating in a parallel manner. At the end of incubation, the cell monolayers were meticulously rinsed four times with a culture broth to eliminate residual unattached bacteria. The entire number of attached bacteria in every chamber was calculated by the lysis of cells using 1 ml of 0.10 % Triton X-100 in water at 37 °C for 5 min. Following this, the survival determination was undertaken on nutrient agar. The ratio of attached bacteria was computed by analogizing CFU with control groups. *L. rhamnosus* PTCC 1637 was chosen as a positive control.

Statistical analysis

All tests were executed three times, and statistics were provided as mean ± SD. All comparisons were conducted via one-way ANOVA and significant variations ($P < 0.05$) among means were examined by Duncan's test procedures by means of SPSS 19 (SPSS Inc., Chicago, IL, USA).

RESULTS

Acid and bile salt tolerance

At first, acid and bile salts tolerance patterns of the isolated *Lactobacillus* were assessed *in vitro*. The rates of survival of the eight *Lactobacillus* isolates were close to 100% at pH 3.0 (Table 1). These outcomes demonstrated that *Lactobacillus* sp. S8 had greater acid resistance than other isolates. Therefore, the uppermost acid tolerance isolates (13 isolates) were opted for bile salts tolerance examination. All chosen isolates were stable in different bile salts concentrations (0.2%-1%) (Table 2). In this context, isolates M10, M16 and S12 demonstrated acceptable tolerance to bile salts at 1% concentration in comparison to other isolates.

Table 1 The results of isolates to acid tolerance.

Isolate	Description	Acid tolerance
D1	Iranian fermented milk isolate	99%
D3	Iranian fermented milk isolate	92%
D9	Iranian fermented milk isolate	83%
M1	Yogurt isolate	79%
M3	Yogurt isolate	0
M8	Yogurt isolate	0
M5	Yogurt isolate	0
M7	Yogurt isolate	80%
M10	Yogurt isolate	99%
M16	Yogurt isolate	98%
M17	Yogurt isolate	98%
S1	Raw milk isolate	0
S7	Raw milk isolate	98%
S8	Raw milk isolate	100%
S9	Raw milk isolate	96%
S11	Raw milk isolate	98%
S12	Raw milk isolate	89%
S14	Raw milk isolate	95%
S15	Raw milk isolate	99%
Y4	Yogurt isolate	0
Y6	Yogurt isolate	81%

Table 2 The results of isolates to bile salts tolerance

	Bile salt tolerance				
	0.2%	0.4%	0.6%	0.8%	1.0%
S14	+4	+3	+3	+2	+1
S8	+4	+3	+3	+2	+1
M17	+4	+3	+3	+2	+1
S9	+4	+3	+2	+2	+1
D1	+4	+3	+3	+2	+1
D3	+4	+3	+3	+2	+1
D9	+4	+3	+3	+2	+1
S12	+4	+4	+4	+3	+2
S7	+4	+3	+3	+2	+1
S11	+4	+3	+3	+2	+1
S15	+4	+3	+3	+2	+2
M16	+4	+3	+3	+2	+2
M10	+4	+4	+4	+3	+2

Adhesion studies of LAB isolates

All the selected isolates were capable of attaching to HT-29 cells; however, there were significant differences between the LAB isolates (Figure 1). The adhesion

levels fluctuated from 3.5% ± 0.60 till 9.9% ± 0.70. In this context, the isolates S8 and S7 exhibited higher adherence (9.3% ± 0.35 and 9.9%±0.70) to HT-29 cell line in comparison to standard strain *L. rhamnosus* PTCC 1637 (10.10 % ± 0.40). The isolates D1, S15 and M10 showed moderate adhesion to HT-29 cell line (5.2% ± 0.51, 5.3% ± 0.50 and 5.1% ± 0.25). Moreover, the isolates S9 and S12 depicted low adhesion feature with per cent adhesion value of 3.5 ± 0.60 and 3.9 ± 0.53 to HT-29 cell line, respectively in comparison to other isolates as well as control culture. All isolates except for S8 and S7, exhibited significant differences (P<0.0001) from the positive control.

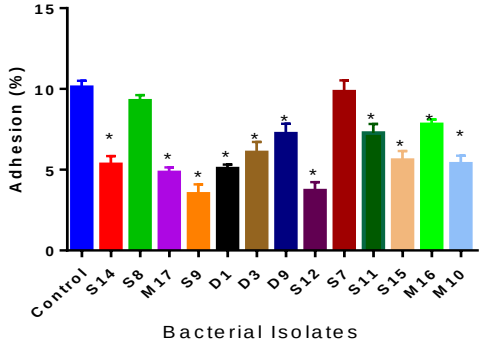

Figure 1 Adhesion of isolates to HT-29 cell lines. *L. rhamnosus* PTCC 1637 is as positive control. The bars show the SD. * showed the significant levels at $P \leq 0.05$ and $P \leq 0.0001$.

Lipase inhibitory assay

The outcomes of rat intestinal lipase inhibitory activity are depicted in Figure 2. The inhibitory activities of bacterial supernatants fluctuated from 33.6 ± 5.7 to 80.55 ± 6.8%, with the topmost value in isolate S11 (80.55 ± 6.8%) followed by isolate S8, D9 and Sa4 (80.55 ± 6.8%, 80.2 ± 6.8% and 80.10 ± 6.8%) which are comparable to *L. rhamnosus* PTCC 1637 75.10 ± 6.4%) as a standard strain as well as orlistat although they were not statistically significant. In contrast, the lowest lipase inhibitory activity was observed for S9 isolate with the value of 35.30 ± 3.2% in contrast to standard strain.

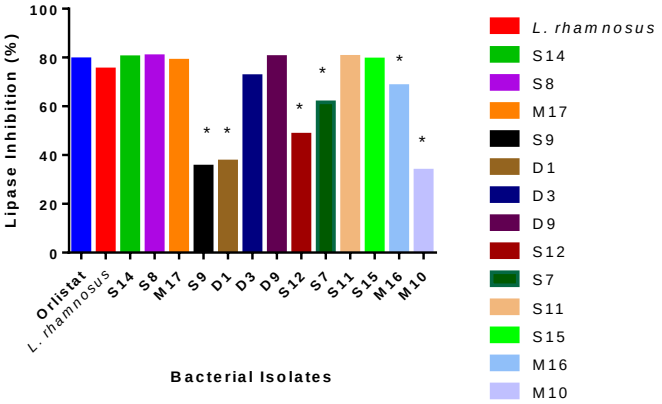

Figure 2 The lipase activity inhibition by selected isolates and *L. rhamnosus* PTCC 1637 as standard strain. Orlistat is as positive control. The bars show the SD. * showed the significant levels at $P \leq 0.05$.

Identification and selection of isolates

A total of 21 Gram positive bacilli-shaped and negative catalase activity bacteria were selected from traditional dairy products. After initial screening for probiotics capabilities, including lipase activity inhibition, further identification was performed. In this regard, the DNA extraction was performed followed by amplification with universal 16s rDNA primers, and each isolates producing a single PCR amplicon of approximately 480 bp. Finally, the individuality of *Lactobacillus* isolates to species level was recognized by DNA sequencing followed by morphological, cultural and biochemical traits using microtechnique method (Table 3). In this context, the isolates named S14, S8, D9, S11 and S15 that had highest lipase activity inhibition were recognized as *L. fermentum* (S14, S8, D9). *L. paracasei* (S11) and *L. brevis* (S15).

Table 3 The biochemical analysis of isolates M2 and M6 using microtechnique approach

	Galactose	Lactose	Arabinose	Cellobiose	Gluconate	Glucose	Melibiose
S14	+	+	+	-	+	+	+
S8	+	+	+	-	+	+	+
D9	+	+	+	-	+	+	+
S11	+	+	-	+	+	+	+
S15	+	+	+	-	-	+	+
	Raffinose	Ribose	Sorbitol	Saccharose	Xylose	Rhamnose	
S14	+	+	+	+	+	-	
S8	+	+	+	+	+	-	
D9	+	+	+	+	+	-	
S11	-	+	+	+	-	-	
S15	+	+	-	+	+	-	

DISCUSSION

Pancreatic lipase is responsible for hydrolyzing lipid triacylglycerol into fatty acids and 2-monoacylglycerols (**Fave *et al.*, 2004; Mohkam *et al.*, 2022**). Orlistat as a common suppressor of pancreatic lipase, possesses outstanding potency and prohibits nearly 30% hydrolysis of the consumed fat; though, orlistat possesses several adverse reactions, including irritability, gastrointestinal illness, bile excretion illness, and hindrance of lipid-soluble vitamin absorption (**Borazjani *et al.*, 2019; Bray, 2009**). Therefore, the pancreatic lipase hydrolysis suppression in the gut is an efficacious tactic for hindrance of hyperlipidemia and obesity. The inhibitors for pancreatic lipase like orlistatare presently administrated clinically as pharmaceutical drugs (**Hill *et al.*, 1999**).Moreover, it has been described that dietary agents, comprising of saponin (**Oishi *et al.*, 2007**), chitosan (**Han *et al.*, 1999**), and oolong tea polyphenols*)* **Nakai et al., 2005***(*, prevent pancreatic lipase, leading to repress of dietary lipid absorption without side effects. In this research, the isolates *L. fermentum* sp D9, *L. fermentum* sp S14 and *L. fermentum* sp S8, *L. paracasei* S11 and *L. brevis* S15, suppressed lipase activity in vitro higher than orlistat and *L. rhamnosus* PTCC 1637 as standard probiotic. In agreement with our outcomes, Zhou et al. (**Zhou *et al.*, 2013**), and Matsumura (**Matsumura, 2010**) depicted that particular *Lactobacillus* strains obstruct pancreatic lipase. There are two mechanisms for inhibition of lipid hydrolysis: a: direct interaction among the repressor and enzyme, b: alteration of lipid emulsion features. It was realized that orlistat prohibited lipase activity using ρ.nitrophenyl.acetate as a substrate, which implies that it has directly suppression effects on enzyme rather than acting on lipid emulsion of substrate. In this regard, the isolated *Lactobacillus* strains had direct lipase inhibition activity that provided well inhibitory impact on lipase activity.

The primary host parameters that may influence probiotic bacteria are the excessive pH level in the stomach and the excessive levels of bile salts in the digestive tract (**Daoudou *et al.*, 2011; Hyronimus *et al.*, 2000**). Consequently, the ability to tolerate acidic environments is a vital gauge for consideration when it comes to selecting potent probiotic strains to ensure their functionality and viability. Furthermore, probiotic bacteria demonstrate varying levels of tolerance to acidic conditions, which is species and strain dependent (**Fontana *et al.*, 2013**). The rates of survival of the eight *Lactobacillus* isolates were close to 100% at pH 3.0 (Table 1). In accordance with our results, Fontana et al. (**Fontana *et al.*, 2013**) stated that *Lactobacillus* spp. isolates demonstrated to be vastly able to withstand acidic conditions, showing superior endurance at pH 3.0 for 1 hr. Instead, researches have demonstrated that *Bifidobacterium* spp. isolates are more vulnerable to acidic conditions than to Lactobacilli spp.. (**Fontana *et al.*, 2013**). In general, LABs are acidophilic, which implies their capability to tolerate acidic circumstances. Nonetheless, this requires to be discriminated from a situation of elevated free acids (H^+) concentration, as the free acids may lead to growth hindrance (**Amrane *et al.*, 2001**). Probiotic bacteria should remain alive during transit through the stomach at a pH of 1.5 to 2.0 (**Dunne *et al.*, 2001**), and sustain themselves for at least 3 hours or longer (**Daoudou *et al.*, 2011**) until they reach the bowel. In this regard, the rate of food transit for humans is slower when compared with other animals, particularly birds; thus, the ability of bacteria to endure acid is a vital factor in humans (**Boonkumklao *et al.*, 2006**). In this context, 13 lactobacilli isolates showed excellent acid tolerance (above 85%) in comparison to others.

Primarily, the ability to tolerate bile salts was regarded as a situation for bacteria to reside in the host and to perform metabolic activities within the bowel (**Havenaar *et al.*, 1992**). They can act as antimicrobial molecules, affecting the intestinal microflora (**Fontana *et al.*, 2013**). Therefore, when assessing the efficacy of the administration of LAB as a probiotic, it is generally imperative to assess their aptitude for tolerating bile salts (**Mohkam, Rasoul-Amini, *et al.*, 2016**).

According to Table 2, out of the isolated Lactobacilli, isolates M10, M16, and S12 had acceptable tolerance of bile salt concentrations of up to 1% at 24 hours of incubation. Bile salt levels in the small intestine are normally between 0.2% and 0.3%, and in some cases, may reach 2% (w/v), depending on the individual and the dietary regime (**Mohkam et al.**, **2016**). In consistent with Mohkam *et al*. (**Mohkam *et al.***, **2020**), tolerance to bile salts differs greatly among the LAB species as well as among strains themselves. The resistant to bile of some strains is correlated to the bile salt hydrolase (BSH) activity that contributes to catalyze conjugated bile, leading to reduction of its toxic influence (**Bustos *et al.***, **2012**). Therefore, BSH activity was the most frequently observed in bacterial isolated from the bowel or feces of human and animals (**Wang *et al.***, **2021**).

The ability of probiotic bacteria to adhere to epithelial cells in the gastrointestinal tract following colonization is a feature that provides an excellent benefit to probiotic bacteria and makes them successfully compete and express ideal functionality, including lipase secretion. Hence, this is an appropriate trait for seeking the isolation of novel probiotic strains. For the determination of adhesion features in vitro, cell cultures of a mucus-excreting HT-29 cell line is the most frequently used (**J. Kim *et al.***, **2015; Sadraeian *et al.***, **2009**). Adhering of bacteria is on the basis of non-specific electrostatic interactions among surfaces, and consequently, it is generally related to the traits of the cell surface. The mucosa of the intestine is distinguished by mucin production, a smarmy glycoprotein that shapes a shielding layer in the intestine. In the current research, the numbers of isolates attaching to the HT-29 cell line were enumerated by colony count on MRS agar. The frequency of adherence to the HT-29 cell line was moderately superb between the strains isolated from the milk and fermented milk samples than those isolated from yogurt; this implied that adhesive LAB isolates have host-residential features distinctive from their populace of origin. Hence, our indigenous LAB isolates will have a surplus favorable impact on the Iranian populace rather than common strains available commercially on the international or national markets, which were in agreement with the previous researches on the adhesion. Adherence of LAB isolates to HT-29 varied between strains and differed within similar species, which was consistent with the findings of **Duary *et. al*** (2011) (**Duary *et al.***, **2011**).

CONCLUSION

We examined whether LAB isolated from the native dairy products illustrate any pancreatic lipase (PL) inhibitory activity. The selected strains were identified as *L. fermentum* sp D9, *L. fermentum* sp S14 and *L. fermentum* sp S8, *L. paracasei* S11 and *L. brevis* S15 by means of the microtechnique biochemical test and16S rDNA sequence. These isolates could survive in harsh gastrointestinal conditions, including bile salts and acid. Moreover, they had an appropriate ability to attach to the HT-29 cell line, which simulates the adhesion to hosts' intestine tract. These findings suggest that these isolates could be considered outstanding strains for anti-obesity activity.

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