Isolation, technological characterization and in vitro probiotic evaluation of *Lactococcus* strains from traditional Turkish skin bag Tulum cheeses

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Abstract

Purpose The present study was undertaken to evaluate in vitro prerequisite probiotic and technological characteristics of ten *Lactococcus* strains isolated from traditional goat skin bags of Tulum cheeses from the Central Taurus mountain range in Turkey.

Methods All isolates were identified based on the nucleotide sequences of the 16S rRNA gene. Eight isolates belonged to *Lactococcus lactis* and two belonged to *Lactococcus garvieae*. Probiotic potential was determined from resistance to acid and bile salt, resistance to gastric and pancreatic juices, resistance to antibiotic, auto-aggregation, co-aggregation, diacetyl, hydrogen peroxide and exopolysaccharide productions. Technological properties were verified by alcohol, NaCl and hydrogen peroxide resistance and temperature tests.

Results *L. lactis* NTH7 displayed high growth at all alcohol concentrations while *L. lactis* NTH4 grew very well even at NaCl concentrations of 10%. All strains showed to some extent resistance to acid and bile. Five strains exhibited desirable survival in gastric juice (pH 2.0), while three strains survived in pancreatic juice (pH 8.0). All *Lactococcus* isolates were sensitive to ampicillin, chloramphenicol, erythromycin, vancomycin, kanamycin, gentamycin and tetracycline. Also, only *L. lactis* NTH7 from among the isolates showed resistance against penicillin. *L. lactis* NTH10 and *L. lactis* NTH7 had higher auto-aggregation values in comparison with all other strains. All the strains demonstrated a co-aggregation ability against model food pathogens, particularly, *L. lactis* NTH10 which showed a superior ability with *L. monocytogenes*. All the ten strains produced H₂O₂ and exopolysaccharide (EPS); however, diacetyl production was detected for only four strains including *L. lactis* NTH10.

Conclusion These results demonstrate that the *L. lactis* NTH10 isolate could be regarded as a favorable probiotic candidate for future in vivo studies.

Keywords Probiotic · Technological characteristics · *Lactococcus* · Tulum cheese

Introduction

Lactic acid bacteria (LAB) are Gram-positive bacteria that naturally exist as endogenous microbiota in raw milk and in other food sources such as vegetables and meat. They play an important role as starter or adjunct cultures for the fermentation of foods. A large number of LAB strains have been

isolated from different fermented foods, and selected strains have been used in industrial food fermentations (Adamberg et al. 2003). LAB have a significant effect on acidification, texture and sensorial properties of fermented foods such as yoghurt, cheese, pickle, sauerkraut, etc. Moreover, LAB constitute a large proportion of probiotic bacteria (Collins and Gibson 1999). Food grade LAB have been investigated for their probiotic potentials and are used as adjunct cultures in various food products (Rodgers 2008).

Probiotics are defined as living microorganisms that provide beneficial health effects to humans when they are consumed in sufficient amounts (FAO/WHO 2001). Potential health effects of probiotic bacteria involve lowering cholesterol and preventing or relieving symptoms of lactose intolerance, irritable bowel syndrome, hypertension, inflammatory bowel diseases, diarrhea and diabetes (Weichselbaum 2009;

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Ooi and Liong 2010). Therefore, foods containing probiotics are referred to as functional foods. The functional food industry has focused on the isolation of new probiotic strains with health-promoting benefits (Umer Khan 2014). In addition to its beneficial health effects, its technological features are also important for industrial use. In vitro tests such as antimicrobial activity, resistance to gastrointestinal conditions, acid and bile salt tolerance, etc., are usually carried out as a first approach (Kumar et al. 2015) in identifying potential probiotic microorganisms that can meet the increasing industrial demand (Das et al. 2016; Abushelaibi et al. 2017; Bengoa et al. 2018).

Tulum cheese is a variety of ripened cheese produced from raw milk. No starter culture is used in its production; thereby, its microbial content consists of the natural or wild microbiota of raw milk. Accordingly, a spontaneous fermentation occurs during Tulum cheese manufacturing. Based on previous studies, spontaneously fermented foods seem to be a good source of new LAB strains with potential probiotic attributes (Takeda et al. 2011; Tulumoğlu et al. 2014). Domingos-Lopes et al. (2017) reported that LAB strains isolated from raw cow milk cheese (Pico cheese) generated diacetyls and exopolysaccharides. In addition to this study, it was found that *Lactococcus lactis* strains isolated from Lighvan cheese, also a raw milk cheese, had antibacterial activity (Attar et al. 2018), while another study found that LAB strains isolated from raw sheep milk cheese were resistant to gastric juice, pancreatic juice and bile salt and had high acid tolerance (Meira et al. 2012).

Previous studies related to the determination of the LAB population of Tulum cheese were carried out for quality standardization and preserving the product characteristics (Gurses and Erdogan 2006; Cakmakci et al. 2008), but probiotic and technological properties of the isolates were not considered in these studies. Tulumoğlu et al. (2014), on the other hand, examined probiotic characteristics of *Lactobacillus* strains isolated from Tulum cheese. However, there are no previous studies in the literature about the probiotic and technological properties of *Lactococcus* strains isolated from Tulum cheese. The aims of this study were to isolate and investigate *Lactococcus* strains from Tulum cheese in order to provide a collection of LAB strains that could be utilized as starter or adjunct cultures for the food industry based on their defined technological and probiotic properties. In this context, diacetyl production, hydrogen peroxide production, exopolysaccharide production, auto-aggregation, co-aggregation, antibiotic resistance, acid tolerance, resistance to bile salt, and resistance to gastric and pancreatic juices tests were applied to assess the probiotic potential of the *Lactococcus* isolates. Moreover, the technological characteristics of the isolates were evaluated using alcohol resistance, NaCl resistance, hydrogen peroxide resistance and growth at different temperatures tests. *Lactococcus* strains isolated from Tulum cheese were identified by 16S rRNA gene sequencing.

Material and methods

Culture conditions

All *Lactococcus* strains were grown in M17 broth (Merck) at 30 °C for 24 h under aerobic conditions. Indicator strains *Escherichia coli* ATCC 25922, *Listeria monocytogenes* ATCC 7644, *Salmonella typhimurium* ATCC 14028, *Staphylococcus aureus* ATCC 25923 and *Bacillus cereus* ATCC 14579 were used for antimicrobial and co-aggregation analyses and were supplied from the Provincial Control Laboratory in Konya, Turkey. All indicator strains were incubated in Brain Heart Infusion broth (Merck) at 37 °C for 24 h under aerobic conditions. Purified isolates and indicator strains were stored at –80 °C by adding 20% sterile glycerol (v/v). The cultures were activated before use.

Isolation of *Lactococcus* strains

Lactococcus strains were isolated using the spread-plate and streaking methods on LM17 medium (Merck) supplemented with 5 g/l of lactose (Randazzo et al. 2006). For this purpose, 10 g of a Tulum cheese sample was diluted in a 90-ml sodium citrate solution (2%) (Merck) and homogenized with a Stomacher (HG400V, Mayo International, Italy). Serial dilutions were made and plated on LM17 medium. The plates were incubated at 30 °C for 48 h under aerobic conditions. After incubation, cultures were gathered randomly from plates. The cultures were then streaked over an agar surface of LM17 medium and incubated again under the same growth conditions. This process was repeated twice, and then, pure cultures were collected. The cultures were checked regularly for purity using a microscope. The pure cultures were transferred to the M17 broth to maintain their purity and stored under the above mentioned conditions.

Molecular identification of *Lactococcus* isolates

Isolated strains were identified by 16S rRNA sequencing. In this context, F365 (5'-ACWCCTACGGGWGGCWGC-3') and R1064 (5'-AYCTCACGRCACGAGCTGAC-3') universal primers, designed from an invariant region in the 16S rRNA sequences for LAB, were used in PCR amplification and were obtained from the Sentegen Biotech Co., Turkey. The PCR amplification was performed in a final 30-μl reaction volume using a BioRAD thermal cycler (T100™, Foster City, California, USA).

For DNA extraction, activated stock cultures were left to incubate in LM17 agar at 37 °C for 48 h under aerobic conditions. At the end of the incubation, single colonies in the LM17 medium were suspended in sterile Eppendorf tubes containing 10 μl of PCR-grade water. A 1-μl aliquot of each sample suspension was transferred to an Eppendorf tube, and

21.3 µl of PCR-grade water, 1.2 µl of MgCl₂, 3 µl of PCR buffer, 1 µl of reverse primer, 1 µl of forward primer, 0.5 µl of Taq DNA polymerase and 1 µl of dNTP solutions were added to produce a final 30-µl PCR mixture volume. The chemicals used were supplied by Thermo Fisher Scientific.

The PCR conditions for the amplification procedure were as follows: initial denaturation at 95 °C for 5 min, 35 cycles of denaturation at 95 °C for 30 s, primer annealing at 58 °C for 30 s and extension at 72 °C for 45 s and one cycle of final extension at 72 °C for 10 min. Presence of PCR products and their purity were verified by agarose gel electrophoresis. The PCR products were sent to Sentegen Biotech (Turkey) for purification and sequencing. *Lactococcus* strains were identified by comparing the sequence results with the DNA sequence database present at the National Centre for Biotechnology Information (NCBI) using the BLAST algorithm (<http://ncbi.nlm.nih.gov/BLAST>).

Strain characteristics associated with probiotic potential

Tolerance to bile salt

Bile salt resistance was determined according to the method of Sabir et al. (2010). An aliquot of 250 µl of bacterial suspension (~9 log CFU/ml) was inoculated into 5 ml of sterile M17 broth and incubated at 37 °C for 24 h. After incubation, bacterial cells were collected by centrifugation (10,000g, 10 min, 4 °C), and then washed thrice and resuspended in a phosphate buffer (PBS). A 0.1 ml of bacterial suspension was added to 0.9 ml of 0.15%, 0.3% and 0.5% bile salt solutions and control solution (i.e. without bile salt) and incubated at 37 °C for 2 h. Following the incubation period, bacterial growth was observed by recording absorbance at 600 nm and results were stated as optical density.

Resistance against acid

Resistance under different acidic conditions was evaluated using a bacterial suspension (~9 log CFU/ml) in sterile distilled water adjusted to pH levels of 1.0, 2.0 and 3.0 by the addition of HCl as defined by Mishra and Prasad (2005). Sterile distilled water with a pH 6.4 was used as a control. Following the pH application, 1 ml of each pH solution was extracted after 2 h and bacterial growth was monitored by measuring absorbance at 600 nm. Results were given as optical density.

Tolerance to gastric and pancreatic juices

Simulated gastric and pancreatic digestions were analyzed using the methods of Bautista-Gallego et al. (2013) with slight modifications. Simulated gastric juice was prepared in a

solution at pH 2.0 containing KH₂PO₄ (0.60 g/l), KCl (0.37 g/l), NaCl (2.05 g/l) and CaCl₂ (0.11 g/l). Prior to use, the solution was sterilized, and then, lysozyme (0.01 g/l) and pepsin (13.3 mg/l) enzymes were added. In the meantime, activated cultures were centrifuged (10,000×g, 10 min) and washed twice with PBS buffer (pH 7.0). The culture pellets were then resuspended into the simulated gastric juice to give a final concentration of 8 log CFU/ml and incubated at 37 °C for 3 h. Serial dilutions of the incubated gastric juices were plated on M17 agar and enumerated after incubation for 3–5 days at 30 °C.

Simulated pancreatic juice was formulated in a solution at pH 8.0 consisting of Na₂HPO₄·7H₂O (50.81 g/l), NaCl (8.5 g/l), pancreatin (0.1 g/l) and bile (3 g/l). For sterilization, the solution was passed through a 0.22-µm membrane filter. At the same time, activated cultures were harvested by centrifugation at 10,000g for 10 min and washed twice with PBS buffer (pH 7.0). The culture pellets were then resuspended into the simulated pancreatic juice to provide a final concentration of 8 log CFU/ml and incubated at 37 °C overnight. After the incubation, serial dilutions of the cultures were plated on M17 and counted following incubation at 30 °C for 3–5 days. The culture pellets resuspended in PBS (pH 7.0) were used as control for these tests. The tolerances of the strains to simulated gastric and intestinal transits were evaluated by comparison with counts of viable cells resuspended in PBS and incubated in gastric and pancreatic juices. Results were expressed as survival rate during gastric and pancreatic digestion.

Antibiotic resistance

Antibiotic resistance of isolates was determined based on the agar disc diffusion method described by Charteris et al. (1998a). *Lactococcus* strains were activated in M17 broth overnight. All isolates were assessed for their susceptibility to ampicillin (10 µg/disc), chloramphenicol (30 µg/disc), erythromycin (15 µg/disc), vancomycin (30 µg/disc), kanamycin (30 µg/disc), gentamycin (10 µg/disc), penicillin (10 IU/disc), streptomycin (10 µg/disc) and tetracycline (30 µg/disc). For evaluating the antibiotic resistance, 100 µl of an activated culture was spread on M17 agar and allowed to get absorbed. Thereafter, commercial antibiotic discs (Bioanalyse) were placed on the inoculated plates with sterile conditions and incubated at 37 °C for 24 h. After incubation, the diameter (mm) of inhibition zones was measured. Results were evaluated as sensitive, intermediate sensitive and resistant considering the zone diameters specified by Khemariya et al. (2013). If the diameter of the inhibition zone was between the range of 10 and 35 mm or smaller than 10 mm, the strain was defined as sensitive or intermediate sensitive, respectively. Also, the absence of the inhibition zone was evaluated as resistant.

Auto-aggregation and co-aggregation

Abilities of the *Lactococcus* strains to aggregate with each other (auto-aggregation) and with pathogens (co-aggregation) were analyzed as detailed by Kos et al. (2003). Activated cultures were gathered by centrifugation at 5000g for 15 min and washed twice in PBS. The harvested cultures were then resuspended in PBS to give viable counts of 8 log CFU/ml. An aliquot of 4 ml of the culture suspension was resuspended by the use of a vortex for 10 s and auto-aggregation was observed during 24 h of incubation at room temperature. At different time intervals (0, 1, 2 and 24 h), 100 µl of the suspension was transferred into another vial containing 3.9 ml of PBS and absorbance (A) was calculated at 600 nm. The auto-aggregation percentage was determined as $1 - (A_t / A_0) \times 100$, where A_t stands for the absorbance at time 1, 2 or 24 h and A_0 stands for the absorbance at time 0.

Co-aggregation of the *Lactococcus* strains against five different pathogens was also assessed. For this purpose, 2 ml of each *Lactococcus* strains was mixed with 2 ml of each pathogen and this bacterial mix was incubated for 24 h at room temperature. At the same time, control tubes were prepared separately containing 4 ml of each bacterial suspension. Samples were taken at different time intervals as in the auto-aggregation assay and absorbance (A) of the suspensions at 600 nm was measured. Co-aggregation percentage was calculated using the following equation:

$$\text{Co-aggregation (\%)} = \frac{((Aa + Ab)/2) - A(a + b)}{(Aa + Ab)/2} \times 100$$

where a and b represent each of the two strains in the control tubes and $a + b$ represents the bacterial mixture.

Exopolysaccharide production

Exopolysaccharide (EPS) production from the *Lactococcus* strains was determined using the method of Van Geel-Schutten et al. (1998). Cultures were inoculated in 20 ml of M17 broth containing 2% glucose (w/v) and incubated for 3 days at 37 °C. Following incubation, the suspensions were centrifuged at 6000g for 20 min to remove the bacterial cells. For precipitation of EPS, a 2:1 volume of cold ethanol (95%, v/v) to supernatant was prepared. EPS precipitates were filtered under vacuum and evaporated at 60 °C. The EPS production from *Lactococcus* strains was determined by measuring the weights of the dried samples. The results were given as positive or negative for EPS production.

Diacetyl production

Activated cultures were centrifuged at 4000 rpm for 15 min, washed with PBS and the pellet was resuspended in PBS. The

bacterial suspension was inoculated (1%) in 10 ml of UHT milk and incubated for 24 h at 30 °C. Thereafter, 1 ml of bacterial suspension was mixed with 0.5 ml of KOH (16%, w/v) and α -naphthol (1%, w/v) solution and subsequently left in incubation at 37 °C for 10 min. Diacetyl production was observed by the occurrence of a red ring at the top of the tubes (Ribeiro et al. 2014). Depending on the red ring formed, the results were expressed as positive or negative in terms of diacetyl production.

Hydrogen peroxide production

Hydrogen peroxide (H_2O_2) production was analyzed spectrophotometrically according to the method of Patrick and Wagner (1949) with some modifications. Active cultures were inoculated (2%) into M17 broth and incubated for 42 h at 35 °C. Following the incubation, 10 µl of culture suspension was mixed with 2.0 ml of hydrochloric acid, 0.2 ml of ammonium molybdate in 1 M sulfuric acid and 0.2 ml of potassium iodide. After the mixture was allowed to stand for 20 min, its absorbance was measured at 400 nm. The same procedures were repeated using H_2O_2 with concentrations ranging from 1 to 10 µg/ml instead of sample to create a standard curve. The H_2O_2 production from *Lactococcus* strains was determined by utilizing the standard curve and the results were evaluated as positive or negative with regards to H_2O_2 production.

Technological characterization

Alcohol resistance

Alcohol tolerance analysis was applied by modifying the method of G-Alegria et al. (2004). Activated cultures were inoculated into a M17 broth supplemented with different ethanol concentrations (3, 6, 12 and 15%) and incubated at 30 °C for 24–48 h. Bacterial growth was assessed by observing turbidity.

H_2O_2 resistance

H_2O_2 tolerance of activated cultures was analyzed using the method of Li et al. (2012). The cultures were inoculated at 1% into M17 broth consisting of 0.4, 0.7 and 1.0 mM H_2O_2 (30%) and incubated for 8 h at 37 °C. Cell growth was determined spectrophotometrically at 600 nm. Results were expressed as optical density.

Growth at different temperatures

Ability to grow at various temperatures was evaluated by modifying the method of Badis et al. (2004). Bacterial growth was assessed visually in M17 broth after incubation for 5 days at 4 °C, 15 °C and 45 °C.

NaCl resistance

Tolerance of the *Lactococcus* cultures towards NaCl was determined according to the modified method of Hoque et al. (2010). Activated cultures were inoculated into M17 broth adjusted with various NaCl concentrations (0, 4, 6.5 and 10%) and incubated at 37 °C for 7 days. After incubation, bacterial growth was evaluated by visual observation.

Statistical analysis

All data were given as the mean and standard deviation of two observations. The results were subjected to one-way ANOVA in Minitab software version 17 (State College, USA) to determine significant differences between means of bacterial cultures and treatments. The means of results were compared with the Tukey test with a confidence interval set at 95%.

Results and discussion

Identification of *Lactococcus* strains by 16S rRNA sequencing

Ten *Lactococcus* strains from Tulum cheese were identified using 16S rRNA sequencing (Table 1). Alignments were performed utilizing the BLAST algorithm. Based on molecular identification, *L. garvieae* NTH1 (99%), *L. garvieae* NTH2 (99%), *L. lactis* NTH3 (99%), *L. lactis* NTH4 (99%), *L. lactis* NTH5 (99%), *L. lactis* NTH6 (99%), *L. lactis* NTH7 (99%), *L. lactis* NTH8 (99%), *L. lactis* NTH9 (99%) and *L. lactis* NTH10 (99%) were determined. Among the identified *Lactococcus* strains, *L. lactis* species was seen to be dominant and it constituted 80% of the designated strains. Likewise, Lopez-Díaz et al. (2000) noticed that *L. lactis* had the highest share in isolates in traditional Spanish cheese.

Tolerance of *Lactococcus* strains at different bile concentrations

Bile salt tolerance is a fundamental parameter for bacterial colonization and viability in the small intestine and colon (Tambekar and Bhutada 2010). Bile salt resistances of isolated *Lactococcus* strains are shown in Table 2. Compared to their relevant control, *L. garvieae* NTH1, *L. lactis* NTH6, *L. garvieae* NTH2, *L. lactis* NTH8 and *L. lactis* NTH10 showed dramatic decrease in their population against increased bile salt concentration ($p < 0.05$). In the meantime, exposure of *L. lactis* NTH3, *L. lactis* NTH4, *L. lactis* NTH5 and *L. lactis* NTH7 to 0.5% bile salt resulted in a significant decrease in their viable cell counts, while lower bile salt concentrations were not effective on their viability. Among the *Lactococcus* isolates, *L. lactis* NTH9 presented relatively high

tolerance to the 2-h incubation under all bile salt concentrations as compared to the others. Moreover, there was no statistically significant decrease in its viable cell count at 0.3% bile salt. Generally, the bile concentration of the intestinal environment is 0.3% (Prasad et al. 1998).

Acid tolerance

The pH of the human stomach varies between 1.5 and 4.5 depending on the food consumption, and food can stay in the stomach for 2–3 h during digestion (Bao et al. 2010). Table 3 shows acid tolerances of ten *Lactococcus* isolates under incubation at pH 1.0, 2.0 and 3.0 for 2 h. *L. lactis* NTH4, *L. lactis* NTH6, *L. lactis* NTH8, *L. lactis* NTH9 and *L. lactis* NTH10 were highly tolerant strains against acid due to their ability to maintain their viable cell counts even in low pH conditions ($p > 0.05$). Kimoto et al. (1999) reported that the growth of the NIAI 527 strain from their *L. lactis* subsp. *lactis* isolates was not affected by low pH. However, in this study, other strains did show variable susceptibility to different pH levels, with a dramatic growth decrease at pH 1.0 ($p < 0.05$). This came as no surprise given the multitude of studies that have observed that low pH levels adversely affect the growth of lactic acid bacteria and that acid tolerance changes from strain to strain (Sabir et al. 2010; Shehata et al. 2016; Abushelaibi et al. 2017).

At pH 1.0, with the exception of *L. garvieae* NTH1 and *L. lactis* NTH3, the growth of all other strains was similar, and their acid tolerances were higher than *L. garvieae* NTH1 and *L. lactis* NTH3. Furthermore, at a pH of 2.0, which mimics the pH of the stomach, the most resistant strain was determined to be *L. lactis* NTH9. Along with the data on bile salt resistance, this result supports the probiotic potential of *L. lactis* NTH9. Although the strain with the highest acid tolerance at pH 3.0 was determined to be *L. lactis* NTH7, the difference between the acid tolerances of *L. lactis* NTH7 and *L. lactis* NTH9 can be considered statistically insignificant. Considering the stomach conditions, resistance against acid is a crucial characteristic for each potential probiotic organism. This feature allows them to be used as dietary adjuncts because they can maintain their viability in foods with high acidity (Chalas et al. 2016).

Survival rates of *Lactococcus* strains during simulated gastric and intestinal transit

The exposure of *Lactococcus* strains to simulated gastric juice for 3 h caused complete loss of viability in the strains with the exception of *L. garvieae* NTH1, *L. lactis* NTH4, *L. garvieae* NTH2, *L. lactis* NTH8 and *L. lactis* NTH10 (Table 4). Accordingly, *L. lactis* NTH3, *L. lactis* NTH5, *L. lactis* NTH6, *L. lactis* NTH7 and *L. lactis* NTH9 strains were detected to be sensitive against gastric conditions.

Table 1 *Lactococcus* isolates identified by 16S rRNA gene sequencing and their GenBank accession numbers adapted from NCBI-BLAST

Isolates	Closest known relative (strain No)	Identification %	GenBank accession No
NTH1	<i>L. garvieae</i> (KE12)	99	HM573320.1
NTH2	<i>L. garvieae</i> (CAU1397)	99	MF429549.1
NTH3	<i>L. lactis</i> (C13)	99	JN942105.1
NTH4	<i>L. lactis</i> (T0625)	99	EU104368.1
NTH5	<i>L. lactis</i> (RPWL3)	99	MF185375.1
NTH6	<i>L. lactis</i> (SNNU0274)	99	KX752895.1
NTH7	<i>L. lactis</i> (NM45)	99	HM218209.1
NTH8	<i>L. lactis</i> (FM19LAB)	99	MF480428.1
NTH9	<i>L. lactis</i> (RCB1008)	99	KT261220.1
NTH10	<i>L. lactis</i> (CAU1157)	99	MF582909.1

L. garvieae NTH1 (27.42%), *L. garvieae* NTH2 (38.97%), *L. lactis* NTH4 (21.23%), *L. lactis* NTH8 (24.56%) and *L. lactis* NTH10 (33.28%) presented appreciable survival rates. Considering that the viable counts of probiotic bacteria exposed to gastric juice were decreased approximately 5–6 log (Vinderola and Reinheimer 2003), the survival rates determined between 21 and 39% against gastric juice is satisfactory in terms of probiotic potential. Moreover, *L. garvieae* NTH2 was evaluated as the most resistant strain to gastric transit. Even though lactococci were reported to be more sensitive than lactobacilli against gastric juice (Faye et al. 2012), the survival rate of *L. garvieae* NTH2 was determined to be higher than the *Lactobacillus fermentum* studied by Charteris et al. (1998b).

Most of the *Lactococcus* isolates were found to be sensitive to intestinal conditions (Table 4). Das et al.

(2016) reported that viable counts of lactic acid bacteria considerably decrease in intestinal juice. However, *L. garvieae* NTH2, *L. lactis* NTH3 and *L. lactis* NTH8 with 27.75%, 17.39% and 19.25% survival rates, respectively, exhibited tolerance to the pancreatic juice. Interestingly, *L. garvieae* NTH2 and *L. lactis* NTH8 showed resistance to pancreatic juice as well as gastric juice, *L. garvieae* NTH2 was detected as the most tolerant strain to pancreatic juice. This case could partially ensure that these strains survive in the gastrointestinal tract. However, performing these tests separately for gastrointestinal transit evaluation has some limitations compared to the continuous system. Therefore, for further evaluation of gastrointestinal conditions, bacteria which exhibited probiotic potential need to be tested in vitro analyses with continuous system.

Table 2 Tolerance of *Lactococcus* strains at different bile concentrations

Isolates	Bile concentration (%)			
	0	0.15	0.30	0.50
<i>L. garvieae</i>				
NTH1	0.109 ± 0.001 ^{ab, a}	0.089 ± 0.007 ^{cd, b}	0.067 ± 0.007 ^{c, c}	0.035 ± 0.005 ^{cd, d}
NTH2	0.096 ± 0.011 ^{c, a}	0.078 ± 0.003 ^{d, b}	0.059 ± 0.001 ^{c, c}	0.044 ± 0.001 ^{c, d}
<i>L. lactis</i>				
NTH3	0.057 ± 0.003 ^{d, a}	0.048 ± 0.007 ^{e, a}	0.048 ± 0.007 ^{d, a}	0.024 ± 0.007 ^{de, b}
NTH4	0.028 ± 0.004 ^{e, a}	0.023 ± 0.002 ^{f, ab}	0.018 ± 0.001 ^{e, ab}	0.009 ± 0.001 ^{f, b}
NTH5	0.024 ± 0.002 ^{e, a}	0.017 ± 0.000 ^{f, ab}	0.011 ± 0.001 ^{e, bc}	0.004 ± 0.000 ^{f, c}
NTH6	0.133 ± 0.007 ^{a, a}	0.098 ± 0.002 ^{bc, b}	0.067 ± 0.002 ^{c, c}	0.033 ± 0.001 ^{cde, d}
NTH7	0.125 ± 0.005 ^{ab, a}	0.108 ± 0.001 ^{ab, ab}	0.087 ± 0.002 ^{b, bc}	0.057 ± 0.002 ^{b, c}
NTH8	0.092 ± 0.008 ^{c, a}	0.081 ± 0.001 ^{d, b}	0.049 ± 0.000 ^{d, c}	0.022 ± 0.000 ^{e, d}
NTH9	0.134 ± 0.018 ^{a, a}	0.118 ± 0.002 ^{a, ab}	0.107 ± 0.002 ^{a, b}	0.094 ± 0.004 ^{a, b}
NTH10	0.026 ± 0.002 ^{e, a}	0.021 ± 0.000 ^{f, b}	0.014 ± 0.001 ^{e, c}	0.008 ± 0.001 ^{f, d}

Results are given as optical density at 600 nm

Different letters in the same row and column are significantly different ($P < 0.05$)

First letters present differences between the strains and second letters demonstrate the differences between the bile concentrations

ns not significantly different

Table 3 Acid tolerances of *Lactococcus* strains at different pH conditions for 2 h

Isolates	Control ¹	pH 1.0	pH 2.0	pH 3.0
<i>L. garvieae</i>				
NTH1	0.056 ± 0.011 ^{d, a}	0.024 ± 0.004 ^{b, b}	0.033 ± 0.009 ^{c, ab}	0.041 ± 0.018 ^{c, ab}
NTH2	0.220 ± 0.021 ^{ab, a}	0.135 ± 0.035 ^{a, b}	0.182 ± 0.017 ^{b, ab}	0.197 ± 0.016 ^{b, a}
<i>L. lactis</i>				
NTH3	0.113 ± 0.017 ^{c, a}	0.022 ± 0.012 ^{b, c}	0.056 ± 0.023 ^{c, bc}	0.071 ± 0.013 ^{c, ab}
NTH4	0.190 ± 0.019 ^{bc, ns}	0.162 ± 0.017 ^{a, ns}	0.188 ± 0.011 ^{ab, ns}	0.211 ± 0.047 ^{ab, ns}
NTH5	0.210 ± 0.022 ^{ab, a}	0.143 ± 0.023 ^{a, b}	0.220 ± 0.019 ^{ab, a}	0.227 ± 0.019 ^{ab, a}
NTH6	0.201 ± 0.027 ^{ab, ns}	0.163 ± 0.019 ^{a, ns}	0.203 ± 0.011 ^{ab, ns}	0.217 ± 0.038 ^{ab, ns}
NTH7	0.274 ± 0.038 ^{a, a}	0.177 ± 0.037 ^{a, b}	0.202 ± 0.022 ^{ab, ab}	0.275 ± 0.022 ^{a, a}
NTH8	0.228 ± 0.045 ^{ab, ns}	0.178 ± 0.041 ^{a, ns}	0.186 ± 0.038 ^{ab, ns}	0.235 ± 0.017 ^{ab, ns}
NTH9	0.239 ± 0.016 ^{ab, ns}	0.207 ± 0.016 ^{a, ns}	0.241 ± 0.016 ^{a, ns}	0.207 ± 0.013 ^{ab, ns}
NTH10	0.217 ± 0.025 ^{ab, ns}	0.187 ± 0.022 ^{a, ns}	0.201 ± 0.013 ^{ab, ns}	0.228 ± 0.015 ^{ab, ns}

Results are given as optical density at 600 nm

¹ Distilled water with a pH 6.4 was used as a control

Different letters in the same row and column are significantly different ($P < 0.05$)

First letters present differences between the strains and second letters demonstrate the differences between the pH conditions

ns not significantly different

Susceptibility to different antibiotics

The determination of antibiotic resistance of strains with probiotic potential is of prime importance. Some bacteria may act as hosts of antibiotic resistance genes, which can be transferred to pathogenic or other bacteria in their environment (Danielsen and Wind 2003). The susceptibility results of *Lactococcus* strains to nine different antibiotics are presented in Table 5. All the *Lactococcus* isolates were found to be sensitive to ampicillin, chloramphenicol, erythromycin,

vancomycin, kanamycin, gentamycin and tetracycline. Likewise, Domingos-Lopes et al. (2017) reported that *L. lactis* and *L. garvieae* strains isolated from raw cow milk were sensitive to chloramphenicol, gentamycin, erythromycin, kanamycin, tetracycline and vancomycin. Besides,

Table 5 Antibiotic susceptibility of *Lactococcus* strains against nine different antibiotics

Isolates	Inhibition zone (mm) ^a								
	Amp	Chl	Ery	Van	Kan	Gen	Pen	Str	Tet
<i>L. garvieae</i>									
NTH1	29	21	17	21	14	14	35	15	30
NTH2	24	21	18	18	12	15	27	10	25
<i>L. lactis</i>									
NTH3	30	27	25	21	12	12	23	13	25
NTH4	31	27	18	21	10	15	35	15	28
NTH5	22	22	19	19	13	16	26	11	25
NTH6	22	22	10	15	11	10	24	7	22
NTH7	26	25	16	20	10	11	0	11	20
NTH8	14	16	12	18	10	25	27	8	24
NTH9	23	21	20	20	10	15	25	20	30
NTH10	25	25	12	16	12	10	25	8	25

Amp ampicillin (10 µg/disc), Chl chloramphenicol (30 µg/disc), Ery erythromycin (15 µg/disc), Van vancomycin (30 µg/disc), Kan kanamycin (30 µg/disc), Gen gentamycin (10 µg/disc), Pen penicillin (10 IU/disc), Str streptomycin (10 µg/disc), Tet tetracycline (30 µg/disc)

^a The values represent the diameter of the inhibition zone (mm). No inhibition zone: resistant, < 10 mm: intermediate sensitive, 10–35 mm: sensitive

Table 4 Survival rates of *Lactococcus* strains during simulated gastric and intestinal transit

Isolates	Survival rate (%)	
	Gastric juice (pH 2.0)	Pancreatic juice (pH 8.0)
<i>L. garvieae</i>		
NTH1	27.42	NG
NTH2	38.97	27.75
<i>L. lactis</i>		
NTH3	NG	17.39
NTH4	21.23	NG
NTH5	NG	NG
NTH6	NG	NG
NTH7	NG	NG
NTH8	24.56	19.25
NTH9	NG	NG
NTH10	33.28	NG

NG no growth

Khemariya et al. (2013) informed that *L. lactis* strains isolated from dairy and non-dairy sources were susceptible to ampicillin and erythromycin but resistant to kanamycin. In contrast with our results, Flórez et al. (2007) stated that *L. lactis* strains isolated from dairy and animal sources showed resistance against tetracycline and streptomycin.

From our antibiotic test results, *L. lactis* NTH7 showed resistance against penicillin, which inhibits cell wall synthesis, where the other strains were sensitive. The resistance to a specific antibiotic may be due to the absence of the target region of the antibiotic in bacterial cell (DeLisle and Perl 2003). On the other hand, *L. lactis* NTH8 and *L. lactis* NTH10 were intermediate sensitive to streptomycin, which is a protein synthesis inhibitor, while the other strains showed susceptibility. For safety aspects, the absence of antibiotic resistance in strains to be used as starter or adjunct cultures is of extreme importance. In this study, most of the *Lactococcus* strains were sensitive to all nine antibiotic tests.

Auto-aggregation ability of *Lactococcus* strains during 24 h (%)

Auto-aggregation values increased from the first hour to the final incubation period (Table 6). The highest auto-aggregation numbers were detected for *L. lactis* NTH7 strain both at the beginning (67.54%) and the end of the incubation period (84.96%) whereas the lowest auto-aggregative strain was *L. garvieae* NTH1 with 3.22% and 19.27% at the initial and last incubation hour, respectively. Four *Lactococcus* isolates showed less than 50% auto-aggregation after 24 h. These are higher than that of an earlier report about *Lactococcus*

strains by Abushelaibi et al. (2017) who documented that auto-aggregations of *L. lactis* KX881768, *L. lactis* KX881782 and *L. garvieae* KX881774 were 2.7%, 25.7% and 9.0% after the 24 h, respectively. In this sense, the difference clearly indicated that auto-aggregation varied greatly amongst even the same species. Nonetheless, in the previous studies, several lactic acid bacteria strains had showed high auto-aggregation percentages such as for *Lactobacillus casei* SM-H (79.8%) and *Pediococcus pentosaceus* VJ41 (89%) (Meira et al. 2012; Vidhyasagar et al. 2013). Aggregation ability is agreed upon as a desirable characteristic of bacterial strains used as probiotics (An et al. 2000). Amongst our *Lactococcus* isolates, *L. lactis* NTH7 had a clear advantage for selection as a probiotic as a result of their strong aggregation capability to intestinal cells.

Co-aggregation ability of *Lactococcus* strains against some pathogenic strains during 3 h

Among these isolates, *L. lactis* NTH10 and *L. lactis* NTH9 effectively aggregate with *L. monocytogenes* at 64.97% and 48.23% after 3 h, respectively. *L. lactis* NTH3 showed the highest aggregation ability with *B. cereus* (38.61%) (Table 7). On the other hand, *L. lactis* NTH8 was the lowest aggregative isolate with *S. aureus* (4.24%) amongst all *Lactococcus* strains. Co-aggregation values varied greatly among strains belonging to the same species and pathogen microorganisms. Maximum aggregation rates were obtained against *L. monocytogenes* whereas the least aggregation rates were against *S. aureus*. Indeed, in a previous study, Vidhyasagar et al. (2013) reported that five *P. pentosaceus*

Table 6 Auto-aggregation ability of *Lactococcus* strains during 24 h (%)

Isolates	Auto-aggregation (%)				
	1 h	2 h	3 h	4 h	24 h
<i>L. garvieae</i>					
NTH1	3.22 ± 0.48 ^{d, c}	3.91 ± 0.63 ^{e, bc}	4.34 ± 0.27 ^{f, bc}	6.41 ± 0.94 ^{f, b}	19.27 ± 2.28 ^{d, a}
NTH2	15.71 ± 1.41 ^{d, c}	18.11 ± 1.63 ^{c, bc}	21.28 ± 1.44 ^{e, ab}	23.39 ± 1.54 ^{e, a}	25.49 ± 2.16 ^{d, a}
<i>L. lactis</i>					
NTH3	38.77 ± 4.27 ^{c, b}	41.21 ± 3.36 ^{c, b}	46.38 ± 5.87 ^{d, b}	51.63 ± 6.32 ^{cd, ab}	64.38 ± 8.77 ^{bc, a}
NTH4	45.85 ± 6.38 ^{bc, ns}	47.33 ± 5.47 ^{c, ns}	48.61 ± 5.51 ^{cd, ns}	51.22 ± 4.28 ^{cd, ns}	59.63 ± 7.68 ^{c, ns}
NTH5	10.25 ± 2.21 ^{d, b}	12.32 ± 1.47 ^{de, b}	14.61 ± 1.96 ^{ef, b}	16.87 ± 3.37 ^{ef, b}	26.94 ± 5.47 ^{d, a}
NTH6	15.62 ± 1.38 ^{d, c}	18.06 ± 2.94 ^{d, bc}	19.42 ± 3.03 ^{e, bc}	24.32 ± 0.98 ^{e, b}	33.66 ± 4.72 ^{d, a}
NTH7	67.54 ± 7.78 ^{a, b}	70.14 ± 5.43 ^{a, ab}	73.47 ± 6.97 ^{a, ab}	77.06 ± 5.18 ^{a, ab}	84.96 ± 4.39 ^{a, a}
NTH8	57.00 ± 4.81 ^{ab, ns}	59.22 ± 3.74 ^{b, ns}	60.38 ± 2.41 ^{bc, ns}	62.17 ± 6.31 ^{bc, ns}	69.47 ± 6.87 ^{abc, ns}
NTH9	40.65 ± 3.56 ^{c, b}	42.04 ± 4.88 ^{c, b}	44.57 ± 5.37 ^{d, b}	46.93 ± 5.07 ^{d, ab}	59.66 ± 6.07 ^{c, a}
NTH10	62.66 ± 5.34 ^{a, ns}	65.34 ± 3.86 ^{ab, ns}	67.08 ± 4.71 ^{ab, ns}	69.84 ± 8.14 ^{ab, ns}	78.09 ± 7.52 ^{ab, ns}

Different letters in the same row and column are significantly different ($P < 0.05$)

First letters present differences between the strains and second letters demonstrate the differences between the times

ns not significantly different

Table 7 Co-aggregation ability of *Lactococcus* strains against pathogenic strains during 3 h (%)

Co-aggregation with <i>B. cereus</i> (%)			
Isolates*	1 h	2 h	3 h
NTH1	12.41 ± 2.16 ^{b, b}	14.32 ± 1.88 ^{b, b}	26.47 ± 3.96 ^{ns, a}
NTH3	27.36 ± 5.22 ^{a, ns}	29.38 ± 4.25 ^{a, ns}	38.61 ± 6.63 ^{ns, ns}
Co-aggregation with <i>S. typhimurium</i> (%)			
	1 h	2 h	3 h
NTH4	17.25 ± 0.69 ^{a, b}	19.72 ± 1.56 ^{a, b}	24.37 ± 2.16 ^{ns, a}
NTH5	11.08 ± 2.51 ^{b, b}	14.16 ± 1.54 ^{b, ab}	20.11 ± 3.43 ^{ns, a}
Co-aggregation with <i>E. coli</i> (%)			
	1 h	2 h	3 h
NTH6	20.34 ± 3.18 ^{a, ns}	24.54 ± 4.73 ^{a, ns}	32.18 ± 5.77 ^{a, ns}
NTH2	7.00 ± 2.11 ^{b, b}	9.12 ± 1.94 ^{b, b}	16.38 ± 4.19 ^{b, a}
Co-aggregation with <i>S. aureus</i> (%)			
	1 h	2 h	3 h
NTH7	7.11 ± 1.33 ^{a, ns}	8.19 ± 2.10 ^{a, ns}	10.34 ± 2.69 ^{a, ns}
NTH8	2.11 ± 0.21 ^{b, c}	3.08 ± 0.52 ^{b, b}	4.24 ± 0.17 ^{b, a}
Co-aggregation with <i>L. monocytogenes</i> (%)			
	1 h	2 h	3 h
NTH9	30.18 ± 2.46 ^{b, b}	37.04 ± 5.78 ^{b, ab}	48.23 ± 6.67 ^{ns, a}
NTH10	52.27 ± 3.74 ^{a, ns}	55.29 ± 2.89 ^{a, ns}	64.97 ± 8.19 ^{ns, ns}

*NTH1 and NTH2 strains belong to *L. garvieae*, NTH3–10 strains pertain to *L. lactis*

Different letters in the same row and column are significantly different ($P < 0.05$)

First letters present differences between the strains and second letters demonstrate the differences between the times

ns not significantly different

isolates showed the highest co-aggregation values (between 86% and 90%) against *L. monocytogenes* compared to the other indicator bacteria. Similarly, Abushelaibi et al. (2017) reported that *L. lactis* KX881768 and *L. lactis* KX881782 showed significantly lower co-aggregation percentages with *S. aureus* after 2 h in comparing with the other pathogens such as *E. coli* 0157: H7, *S. typhimurium* and *L. monocytogenes*. Co-aggregation represents an obstacle to preserve intestine wall surface colonization of pathogenic bacteria, thus advantageous in supporting instead the adherence of beneficial microorganisms. The adhesion of lactic cultures to intestine epithelium is a prerequisite characteristic for probiotic bacteria. Among our isolates, *L. lactis* NTH10 and *L. lactis* NTH9 complied with this criterion better than the other tested *Lactococcus* strains.

EPS, diacetyl and H₂O₂ production of *Lactococcus* strains

Based on EPS test, all *Lactococcus* isolates were positive for EPS production in the present study (Table 8). This result is in

an agreement with those reported by Abushelaibi et al. (2017) who indicated a satisfactory EPS producing ability for three tested *Lactococcus* isolates (*L. lactis* KX881768, *L. lactis* KX881782 and *L. garvieae* KX881774) in their study. However, results from our study contradicted a previous study executed by Franciosi et al. (2009) who claimed that none of their 30 *Lactococcus* isolates could produce EPS. Exopolysaccharides have significant commercial value by means of their industrially useful physico-chemical features; hence, EPS-forming bacteria play an important role in the rheology and texture of fermented food products (Patel et al. 2012). In this sense, EPS-forming capability is taken into consideration in the selection of starter and adjunct cultures, so all these *Lactococcus* isolates have a considerable industrial use potential in the manufacturing of several dairy products.

In tests for diacetyl production, only four *Lactococcus* isolates (*L. garvieae* NTH1, *L. garvieae* NTH2, *L. lactis* NTH7 and *L. lactis* NTH10) exhibited the ability to produce diacetyl (Table 8), and this is in accordance with a previous study which displayed diacetyl production, and the amount of *Lactococcus* isolates was strain-dependent (Franciosi et al. 2009). These researchers observed that only ten isolates produced diacetyl amongst 30 *Lactococcus* isolates. On the other hand, Kondrotiene et al. (2018) reported that none of the 12 *Lactococcus lactis* isolates were able to produce diacetyl. Diacetyl is a volatile compound produced by some LAB as an end product of citrate metabolism and directly linked to good aroma formation. Thus, these four *Lactococcus* isolates can be used for the application as starter or adjunct cultures.

Regarding H₂O₂ production, all *Lactococcus* isolates used in this study generated different amounts of H₂O₂ from each other (Table 8). Several researchers have reported different findings with regard to H₂O₂ production of various *Lactococcus* strains, such as Yüksekdağ et al. (2004) who determined that three out of four isolates of *L. lactis* were

Table 8 EPS, diacetyl and H₂O₂ production of *Lactococcus* strains

Isolates	EPS production	Diacetyl production	H ₂ O ₂ production
<i>L. garvieae</i>			
NTH1	+	+	+
NTH2	+	+	+
<i>L. lactis</i>			
NTH3	+	–	+
NTH4	+	–	+
NTH5	+	–	+
NTH6	+	–	+
NTH7	+	+	+
NTH8	+	–	+
NTH9	+	–	+
NTH10	+	+	+

“–” negative, “+” positive

H₂O₂ producers. It has also been observed that 21 out of 77 *Lactococcus* isolates from Andalusian raw goat milk were able to produce H₂O₂ (Picon et al. 2016). H₂O₂ supports bactericidal activity to prolong shelf life, delays/contrasts food spoilage and diminishes pathogenic microorganisms and their detrimental effects. H₂O₂ produced by LAB might be, therefore, an important part of the fermentation process. Considering the H₂O₂ production of the tested *Lactococcus* strains, all isolates are good candidates for commercial culture use.

Growth studies

The ability of lactic cultures to survive in adequate amount after subjected to low or high temperatures is an important feature for their use in the food industry. Survival at 15 and 45 °C of tested *Lactococcus* strains changed mostly due to strain specificity; however, no growth was observed for all tested strains at 4 °C (Table 9). *L. lactis* NTH8 showed greater growth (++) among the four *Lactococcus* isolates which were able to grow at 45 °C while all tested isolates revealed similar growth at 15 °C (+). The low percentage of lactococci that were able to grow at 45 °C is in agreement with results by Franciosi et al. (2009) which stated that 2 out of 14 *L. lactis* subsp. *lactis* isolates, 1 isolate out of 11 *L. lactis* subsp. *cremoris* and 2 out of 5 *L. garvieae* isolates displayed growth at this temperature. Badis et al. (2004) also revealed that all 46 *L. lactis* and 2 *L. garvieae* isolates exhibited no growth at 45 °C.

Most strains exhibited high growth in the presence of ethanol with the exception of *L. lactis* NTH10 which demonstrated no tolerance above 6% alcohol concentration (Table 9). Among the isolates tested in this study, *L. lactis* NTH7 strain had the highest ethanol resistance; surprisingly, this isolate grew very well even at high alcohol concentrations of 12%, thereby demonstrating the potential of *L. lactis* NTH7 to resist ethanol. When determining a durable microorganism to harsh conditions for production of ethanol, it is judicious to choose one that shows a higher tolerance to ethanol. Generally, ethanol tolerance of lactic acid bacteria (LAB) is known to be high, but previous studies were commonly executed using *Lactobacillus* strains, for this reason limited data exists regarding *Lactococcus* strains and their ethanol tolerance. In a recent study, Hviid et al. (2017) claimed that high concentrations of ethanol were cytostatic to *L. lactis*. While Solem et al. (2013) determined that the wild-type *L. lactis* strain MG1363 grew, although slowly, in the presence of 6% ethanol and mutant derivative *L. lactis* strain MG1363 were capable of growth in the presence of 8% ethanol.

All *Lactococcus* isolates tolerated up to 10% salt and generally displayed high growth in all NaCl concentrations used in this study, whereas the highest growth was observed in *L. lactis* NTH4, even in the presence of 10% salt (Table 9).

These findings indicated that *Lactococcus* isolates in the present study are likely to grow best under high salt concentrations and have the potential for use in manufacturing of several cheeses containing high salt levels, especially *L. lactis* NTH4. Our results are in contrast with that of Cogan et al. (1997) and Karakas-Sen and Karakas (2018) who revealed that some wild-type *L. lactis* subsp. *lactis* strains grew weakly in media consisting of 6.5% NaCl. On the other hand, our results are in an agreement with those reported by de Almeida Júnior et al. (2015) who claimed that 9 out of the 13 *L. lactis* isolates were able to produce good growth at 6.5% NaCl. Obis et al. (2001) claimed that several strains of *L. lactis* subsp. *lactis* and *cremoris* grew well in 6.5% (w/v) salt concentrations, but growth of some other strains was negatively affected even at 2% (w/v) NaCl. In this sense, the differences in results could have been caused by the different strains used in all studies.

Regarding H₂O₂ resistance, growth of *Lactococcus* strains in M17 broth decreased with the increasing exposure ratio to H₂O₂ (Table 9). This is not surprising since oxidative stress often may result in DNA and cell membrane damage and weaken the bacteria. *Lactococcus* strains like other lactic acid bacteria are very sensitive to H₂O₂ because they lack the catalase activity to reduce H₂O₂ into water and oxygen (Rochat et al. 2005). There is an obvious consensus about hydrogen peroxide as an important inhibitor for lactococci (Piard and Desmazeaud 1992). Among all *Lactococcus* isolates, the highest H₂O₂ tolerance was displayed by *L. lactis* NTH8, *L. lactis* NTH5 and *L. garvieae* NTH2, whereas the most vulnerable isolate to all H₂O₂ levels was *L. lactis* NTH3. Thus, this tolerance was observed to be strain-dependent. Similar results were also noted earlier by Piard and Desmazeaud (1992), who stated that autoinhibition by H₂O₂ is not universal among *Lactococcus* strains.

Conclusions

The present study was undertaken to identify suitable probiotic *Lactococcus* strains isolated from traditional goatskin casings of Tulum cheeses from the Central Taurus mountain range in Turkey. These results are a precursor to future in vivo trials. Regarding acid tolerance, antibiotic susceptibility, survival rates during simulated gastric juice, auto-aggregation and co-aggregation ability scores, EPS, diacetyl and H₂O₂ production *L. lactis* NTH10 exhibited satisfactory in vitro probiotic properties and had a good potential to be used as a probiotic microorganism. Further studies should be required to clearly claim *L. lactis* NTH10 as a probiotic strain and focus on improving the technological characteristics for industrial applications.

Table 9 Technological characteristics of *Lactococcus* strains

Characteristics		Isolates**									
		NTH1	NTH2	NTH3	NTH4	NTH5	NTH6	NTH7	NTH8	NTH9	NTH10
<i>Alcohol:</i>											
3%	+	++	++	++	++	++	+++	+++	++	+	+
6%	+	+	+	++	++	++	+	+++	+	+	+
12%	+	+	+	+	++	++	+	+++	+	+	–
15%	–	+	+	+	–	++	+	+++	+	+	–
<i>Growth:</i>											
4 °C	–	–	–	–	–	–	–	–	–	–	–
15 °C	+	+	+	+	+	+	+	+	+	+	+
45 °C	–	+	+	+	–	–	–	–	++	+	–
<i>NaCl:</i>											
4%	+	++	++	++	+++	++	++	++	++	++	++
6.5%	+	++	++	++	+++	++	++	++	++	++	++
10%	+	++	++	++	+++	++	++	++	++	++	++
<i>H₂O₂ resistance</i> *											
0.4 mM	0.454 ± 0.124 ^{c, a}	1.584 ± 0.228 ^{ab, ns}	0.397 ± 0.046 ^{c, a}	0.452 ± 0.103 ^{c, a}	1.840 ± 0.419 ^{a, ns}	1.383 ± 0.347 ^{ab, ns}	1.816 ± 0.469 ^{ab, ns}	1.665 ± 0.336 ^{ab, ns}	0.961 ± 0.177 ^{abc, a}	0.896 ± 0.539 ^{bc, ns}	ns
0.7 mM	0.189 ± 0.066 ^{c, b}	1.336 ± 0.168 ^{a, ns}	0.244 ± 0.121 ^{c, ab}	0.191 ± 0.112 ^{c, b}	1.463 ± 0.356 ^{a, ns}	1.114 ± 0.249 ^{ab, ns}	1.442 ± 0.171 ^{a, ns}	1.320 ± 0.286 ^{a, ns}	0.711 ± 0.092 ^{bc, a}	0.689 ± 0.187 ^{bc, ns}	ns
1 mM	0.147 ± 0.036 ^{c, b}	1.092 ± 0.183 ^{a, ns}	0.121 ± 0.081 ^{c, b}	0.115 ± 0.063 ^{c, b}	1.060 ± 0.243 ^{a, ns}	0.936 ± 0.129 ^{ab, ns}	1.003 ± 0.321 ^{ab, ns}	1.177 ± 0.168 ^{a, ns}	0.329 ± 0.119 ^{c, b}	0.531 ± 0.089 ^{bc, ns}	ns

Maximum growth is demonstrated as three positive signs (+++), high growth is indicated as two positive signs (++) , weak growth is presented as single positive sign (+) and no growth is demonstrated as negative sign (–)

* Results are given as optical density at 600 nm

** NTH1 and NTH2 strains belong to *L. garvieae*, NTH3–10 strains pertain to *L. lactis*

Different letters in the same row and column are significantly different ($P < 0.05$)

First letters present differences between the strains and second letters demonstrate the differences between the concentrations of H₂O₂

ns not significantly different

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Research involving human participants and/or animals Not applicable.

Informed consent Informed consent was obtained from all individual participants included in the study.

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