Indian J Dairy Sci 78(4): 303-312
https://doi.org/10.33785/IJDS.2025.v78i04.002

RESEARCH ARTICLE

Isolation and identification of exopolysaccharides-producing lactic acid bacteria from conventional Egyptian dairy products

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Received: 02 February 2025 / Accepted: 05 March 2025 / Published online: 23 August 2025

Abstract: Previously, lactic acid bacteria (LAB) with noteworthy functional attributes have been reported as conceivable probiotic candidates. Isolation and identification of novel exopolysaccharides-producing LAB from conventional Egyptian dairy products (Laban Rayeb, Kareish cheese, Labneh, baramily cheese, traditional yogurt, and cheese whey) and the probiotic properties of strains were studied. Isolated five LAB strains were identified as *Lacticaseibacillus paracasei* (S1 and S3), *Enterococcus hirae* S2 and *Enterococcus durans* S4. *Enterococcus hirae* S5 and *Lacticaseibacillus paracasei* S3 showed the highest exopolysaccharides yield ability. *Enterococcus hirae* S2 and S5 and *Lacticaseibacillus paracasei* S3 strains appeared high tolerance to low pH, bile salt, and artificial gastric juice. Some identified strains exhibited resistance and the others were sensitive to a wide range of antibiotics except *Enterococcus hirae* S5 that showed sensitive to all tested antibiotics. A cold storage had a positive effect on the viable count of *Enterococcus durans* S4. Our findings revealed that *Lacticaseibacillus paracasei* S3 and *Enterococcus hirae* S2 strains might be suitable probiotic candidates for producing functional food products.

Keywords: Exopolysaccharides, Lactic acid bacteria, Probiotic criteria, Egyptian dairy products.

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Introduction

Recently, there are new trends in the food industry through the development of functional food products with decreasing utilization of non-natural components. Depending on these trends, the quantity of novel research concerning natural functional components seeking to meet the characteristics for functional food products are growing to suit the modernistic consumers. Probiotic bacteria, prebiotics, and postbiotic byproducts are the most used ingredients as bioactive components for the evolution of functional food products. Lactic acid bacteria (LAB) are closely correlated to all these items, pointing out the significance of studying these bacteria. Additionally; they can yield beneficial components to enhance the characteristics and safety of food products (Moradi et al. 2021). LAB are known as generally recognized as safe (GRAS, Shehata et al. 2016) and are considered the major microbial group involved in the fermented food industry (Abdul Hakim et al. 2023). Previously, LAB are vastly utilized as probiotics that is known live microorganisms that have health benefits host when ingested in an adequate quantity (Munir et al. 2022). Now, probiotic bacteria have wide applications in humans and animals, particularly in the control or prevention of different diseases and disorders including cancer, diabetes, allergy, hypertension, fatness, and boosting the immune system (Abdul Hakim et al. 2023).

It is noteworthy that various LAB used in the fermented food industry exhibit diverse metabolic properties procuring different portfolios of biocomponents (Lee et al. 2021). Microbial polysaccharides, particularly exopolysaccharides (EPS) are one of the most important bioactive components that are produced by LAB. There are different genera of LAB are recognized as EPS-producers including *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Pediococcus*, *Streptococcus*, and *Weissella* (Aguirre-Garcia et al. 2024). Nowadays, EPS possesses essential concern because of their broad applications in the food, cosmetic, and pharmaceutical industries (Moradi et al. 2021). EPS produced by LAB exhibit natural substitutes to commercial food additives due to their physical and chemical properties (Ispirli and Dertli 2018). They improve the rheological properties of dairy products including viscosity, texture, microstructure, and water-holding capacity which act as stabilizers, thickeners, emulsifiers, and fatreplacers. Also, EPS might be applied in the preparation of edible coatings (Nguyen et al. 2020; Moradi et al. 2021). Furthermore, EPS can protect and make bacterial cell wall more resistant to toxic components including detergents, nisin, lysozyme, antibiotics, and bacteriophages and these characteristics contribute to the expression of probiotic criteria of the host cells (Bhandary et al. 2023). Korcz et al. (2018) reviewed that LAB-derived EPS have different health benefits due to their putative antibacterial, anti-inflammatory, antitumor, immune-stimulating, and cholesterol lowering activities. Thence, the screening and isolation of novel EPS-producing LAB strains are a worthy manner of attention to preserve biodiversity and to discover novel EPS for the application in the food industry and research targets.

In Egypt, different traditional fermented food products, following professional practices, such as Kariesh cheese, Laban Rayeb, Kishk, and Mish cheese have been homemade-produced and consumed for prolonged in the rural regions (Todaro et al. 2013; Abou-Zeid 2016). Conventionally, Egyptian fermented milk products are manufactured locally from raw or heat-treated milk at small scale and are mostly prepared on site by hand (Mahgoub 2018). Therefore, these products are rich in a community of microorganisms particularly LAB; thus, they remain natural food sources to discover novel strains with healthy and technological characteristics. Hence, the present study was designed to isolate and identify new LAB that produces EPS from traditional dairy products and to describe the probiotic criteria of isolated strains.

Materials and Methods

Materials

de Man, Rogosa and Sharpe (MRS) broth medium was procured from Oxoid Limited company (Hampshire, United Kingdom).

Isolation of EPS-releasing LAB

Twenty-three conventional Egyptian fermented milk products including Kareish cheese (n = 4, cheese was made from skim milk by fermentation), Laban Rayeb (n = 2, fermented milk), Labneh (n = 3, concentrated yogurt), baramily cheese (n = 5, cheese was made from raw milk or pasteurized milk and stored for about 6 months at refrigerator), traditional yogurt (n = 1, fermented milk), and cheese whey (n = 8, a byproduct of cheese manufacturing) were collected from several districts in Giza governorate, Egypt. Aseptically, all samples were collected in sterile bags, kept in an ice-box vessel, fetched to the experimental laboratory, and preserved at 4°C. Under aseptic conditions, each sample (10 g) was weighed in a sterilized bottle containing 90 ml sterilized saline (0.9%) and was mixed well. The suitable ratio of diluent was chosen, then 0.1 ml was inoculated to MRS agar medium and incubated at 37°C for 2-3 days. According to a microscopic examination, negative catalase reaction, and Gram staining, single colonies were picked out and were streaked on to MRS agar medium for precursory identification. All isolated bacteria strains were preserved at -80°C in MRS medium including 50% sterilized glycerol.

Molecular identification of isolated LAB strains

At the beginning, genomic DNA was extracted from various isolated bacterial strains as shown in the DNA extraction kit scheme with a little of changes, as illustrated by Elsayed et al. (2021). Bacterial colonies were harvested from MRS agar-petri dishes and washed 3 times utilizing 0.85% NaCl solution. Deoxyribonucleic acid (DNA) was extracted from isolated strains utilizing the Qiagen DNA extraction Kit (Qiagen, Hilden, Germany) silica bead extraction Kit for DNA (Thermo Scientific, St. Leon-Rot, Germany). DNA extracts were examined and kept at -20°C.

By sequencing the gene coding for 16S rRNA, identification of the five isolates was implemented. 16S rRNA gene amplification was performed utilizing the polymerase chain reaction (PCR, Eppendorf, Germany) through utilizing the global particular forward and counter oligonucleotide PCR primers (Sigma-Aldrich); 28 F 52 AGAGTTTGATCCTGGCTCAG-32 (positions 8-28 in *E. coli* numbering) and 1512 R 52 ACGGCTACCTTGTTACGACT-32 (positions 1512- 1493 in *E. coli* numbering). PCR reaction blend (25 µl) composed of 0.5 µl of forward and counter primer each (20 pmol/µl), 0.5 µl dNTP, 2 µl of model, 2.5 µl 10X buffer, 2.5 µl magnesium chloride, 0.2 µl Taq polymerase and 16.3 µl PCR-water. The PCR outputs were assessed using electrophoresis on 1% agarose gel (80-120 V, 1.5 h, TAE buffer). Utilizing a gel extraction kit (Real Genomics, RBC, India), the PCR magnified-16S rRNA gene output was refined. In addition, the refined PCR magnified DNA was sequenced (Macrogen, Korea). Utilizing the Basic Local Alignment Search Tool (BLAST) algorithm, 16S rRNA gene series of chosen bacterial isolates was matched with the reference sequences ready in the NCBI database. Utilizing the Clustal W software, exceedingly concerned series were restored and ranged. In Fig. 1, the phylogenetic tree was established from developmental distance matrix operation utilizing MEGA 7.0 program (Kumar et al. 2016).

Exopolysaccharides yield

The EPS produced from isolated LAB strains was quantified corresponding to Wu et al. (2010). Isolated LAB strains were cultivated in both MRS broth medium and in adjusted MRS broth medium in which glucose was substituted with lactose. The cultures were centrifuged at 12000 x*g* for 10 min, then the obtained filtrates were mixed with two quantities of chilled ethanol and permitted to stand at 4°C for 12 h to precipitate. The resulted precipitate was separated through centrifugation (5000 x*g* for 10 min) and resuspended in distilled water, followed by mixing of two quantities of chilled ethyl alcohol. The obtained pellets were separated by re-centrifugation and air dried and weighed (EPS amount).

Temperature sensitivity

A sensitivity to temperature was followed by the inoculation of isolated LAB cultures (1%) in 10 ml MRS broth medium and incubated at 15, 32 or 40°C for 48 h. The survival of bacteria was then noted by visual examination for the test tubes.

Salt tolerance

The salt tolerance of isolated LAB strains was investigated as described by Tambekar and Bhutada (2010). The selected LAB strains were inoculated in 10 ml MRS broth medium with different concentrations of NaCl (1, 3, 5 and 7%) and were incubated at 37°C for 48 h. The bacterial growth was noted by visual observation of the test tubes.

Probiotic criteria for identified LAB strains

Acid tolerance

For the studying of acid tolerance of isolated LAB strains, the pH of MRS broth medium was adapted at 2.0 and 3.0 using HCl 1.0 N. Thereafter, the adapted MRS broth medium was pollinated with 1% overnight-cultures of isolated LAB and incubated at 37°C. The samples were taken at zero, 120 and 180 min. The viability of LAB strains was spectrophotometrically determined at 620 nm.

Bile salt tolerance

LAB isolates were examined for their capability to grow in the existence of bile salt (sodium taurocholate) at 0.2, 0.3 and 0.5% (w/v) according to Kuda et al. (2016). The bile salt ratios were fitted in 20 ml MRS broth medium. Prepared MRS broth medium was inoculated with 1% overnight-cultures and incubated at 37°C for 3 h. The survival of LAB isolates was spectrophotometrically determined at 620 nm after 120 and 180 min of incubation period.

In vitro gastric juice tolerance

Isolated strains were tested against simulated gastric juice through the inoculation of gastric juice (MRS broth medium and 3 mg/ml pepsin, pH at 2.0) by 1% overnight cultures and then incubation at 37°C for 3 h. The viability of LAB strains was spectrophotometrically determined at 620 nm (Zhang et al. 2016).

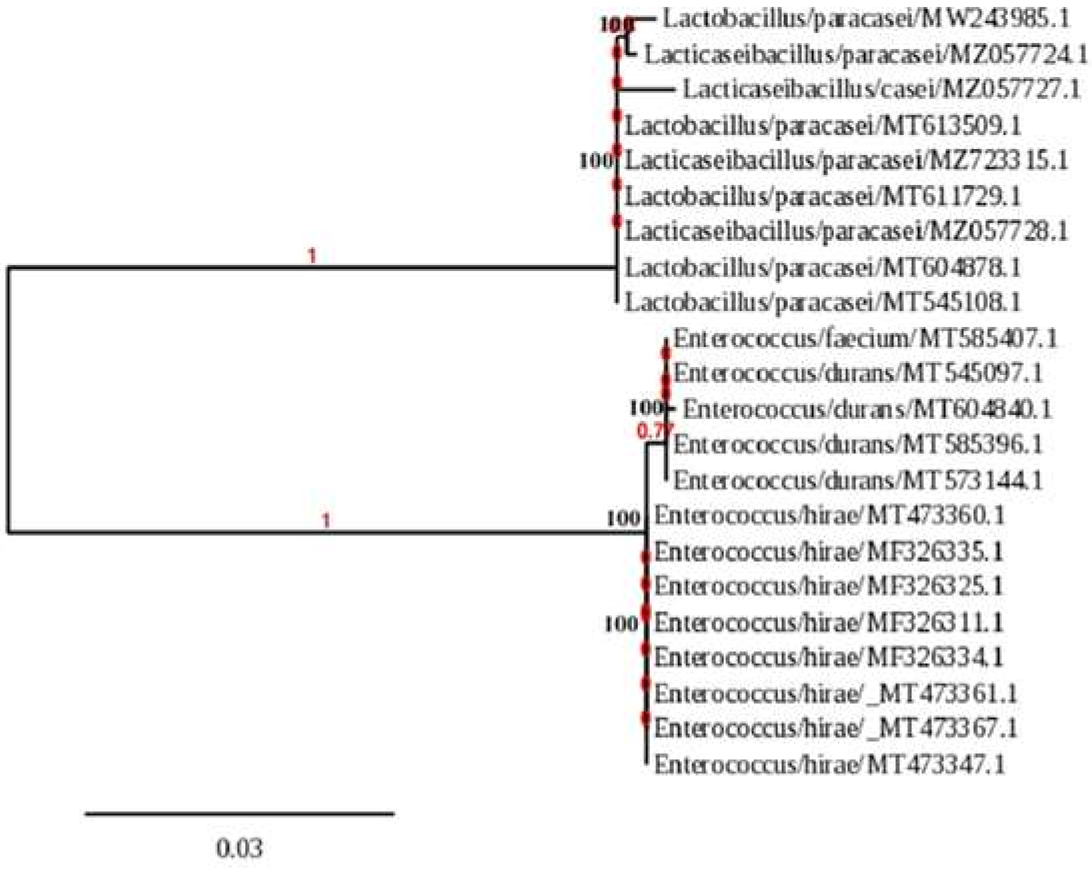


Fig. 1 Phylogenetic tree of isolated lactic acid bacteria and related genera depend on 16S rRNA sequence

Antibiotic resistance

The antibiotic resistance of isolated LAB was examined by agar disc diffusion procedure as described by Sharma et al. (2021). A freshly LAB strain culture (0.1 ml) was blended to 10 ml MRS agar in each plate. On the solidified MRS agar surface, antibiotic discs were put, then the MRS agar plates were incubated for 48 h at 37°C. The isolated LAB strains were tested against the antibiotics of tetracycline (30 µg), vancomycin (30 µg), penicillin (10 µg), lincomycin (15 µg), erythromycin (15 µg) and chloramphenicol (25 µg). Isolated LAB were classified utilizing the cut-off points presented by the CLSI essay (CLSI 2016) and the inhibition zone was recorded in millimeters.

The viability of identified LAB during cold storage

Reconstituted skim milk was sterilized at 115°C for 10 min, followed cooling to 37°C and was inoculated by 1% active culture of identified LAB stains, individually, and then incubation for 24 h. Samples of fermented milk were conserved at 4°C for two weeks.

Statistical assessment

All parameters are assessed in triplicates and the results were shown as means ± standard deviation (SD). Utilizing ANOVA test through MSTAT-C program (Michigan, USA), the data were tested. The variations among means were considered significant at probability of lower than 0.05 confidence level.

Results and Discussion

Forty bacterial strains were isolated from 23 conventional Egyptian milk products, and all of them were investigated for morphological, physiological and EPS yield properties (data not shown). Only 5 isolated strains were selected based on Gram staining (G^+), catalase reaction (Cat^-), and highly producing to EPS. These strains including S1, S2 and S4 were isolated from cheese whey while S3 and S5 strains were isolated from Laban Rayeb. These selected strains exhibited better growth ability at 32°C compared to 40°C and they were well grown at 5% salt except S1 and S4 strains. These five isolated strains were further identified and tested for probiotic characteristics.

Production of exopolysaccharides

Bacterial EPS have remarkable physiological functions to avoid microorganisms from undesirable conditions such as bacteriophages, osmotic stress, antimicrobial substances, and antibodies. Furthermore, microbial EPS are used in the food industry for improving the rheological characteristics of products or for promoting the health potentials (Silva et al. 2019; Shankar et al. 2021). The capability of isolated LAB to produce EPS is shown in Fig. 2A. Selected five strains of LAB exhibited the ability to produce EPS whether in MRS broth supplemented with lactose or glucose. Also, the highest EPS yields were observed in the case of using lactose for all isolated strains. *Enterococcus hirae* S2 significantly showed the highest EPS yield followed by *Lacticaseibacillus paracasei* S3, *Enterococcus hirae* S5, *Lacticaseibacillus paracasei* S1, and *Enterococcus durans* S4. Therefore, these strains can be used as adjunct cultures to produce fermented milk products with enhancing their technological properties. In this respect, Nami et al. (2019) isolated four strains of *Enterococcus durans* and *hirae* from different artisanal dairy products and found all strains had EPS production ability. Several studies reported that *Lactobacillus paracasei* strain could be able to produce EPS (Bibi et al. 2021). Lee et al. (2022) isolated 225 *Lactobacillus* sp. from human feces and they found only *Lacticaseibacillus paracasei* EPS DA-BACS that produced EPS with a high amount.

Identification of selected LAB strains

The molecular relevance among S1, S2, S3, S4 and S5 strains and other exceedingly similar LAB species was shown through a Phylogenetic sequencing depending on 16S rRNA genes (Table 1). The molecular identification exhibited that S1 and S3 strains were akin to *Lacticaseibacillus paracasei*, while S2 and S5 strains were identified as *Enterococcus hirae,* and S4 was closest to *Enterococcs durans* with level of identity of 100% for all strains. In this respect, Abosereh et al. (2016) isolated and identified two strains of *Lactobacillus paracasei* Lb2 and *Enterococcus faecium* En 4 from traditional Egyptian fermented dairy products (Kareish cheese and Kishk) and they proved that two strains were considered as probiotic bacteria. Also, El-Ghaish et al. (2015) identified *Enterococcus hirae* IM1 strain that had antimicrobial activity from Egyptian dairy products, and they pointed out that

Table 1: Molecular identification of 16S rRNA gene sequences generated from isolates

Strains	Accession number Genbank	Sequence length (bp)	Closely related genus/species from GenBank	Level of identity (%)
S1	OL824829	1159	*Lacticaseibacillus paracasei*	100
S2	OL824830	1472	*Enterococcus hirae*	100
S3	OL824831	1495	*Lacticaseibacillus paracasei*	100
S4	OL824832	1443	*Enterococcus durans*	100
S5	OL824833	1479	*Enterococcus hirae*	100

it may be utilized as starter culture or co-culture in the manufacturing of fermented food.

Probiotic criteria of identified strains

Acid tolerance

The identified bacteria strains resistance to acidic environment is one of the main characteristics for probiotics. The probiotic bacteria should be resistant to high acid levels (pH up to 2) that are similar to human stomach conditions (Das et al. 2016). The impact of acid condition (pH 2.0 and 3.0) for 3 h on the survival of identified LAB strains is illustrated in Fig. 3A and Fig. 3B. At pH 2.0, the viability of *L. paracasei* S1 and *Ent. durans* S4 strains significantly decreased after 2 h (survival rates were 82.14 and 93.69%, respectively) while *L. paracasei* S3 significantly decreased after 3 h with survival rate 90.97%. However, the viability of *Ent. hirae* S2 and S5 strains significantly decreased after 3 h of incubation. Regarding the survival at pH 3.0, all strains grew well for 3 h and the survival rate was in the range 91.08-97.78%. These results indicated that the resistance of these strains to gastric stress and utilization of the isolated strains as adjunct cultures in sour food products such as yogurt and Kareish cheese. Malilay et al. (2024) isolated and identified *Lactobacillus paracasei* BIOTECH 10363 strain and they found that it can be grown at low pH for 18 h. Moreover, Nami et al.

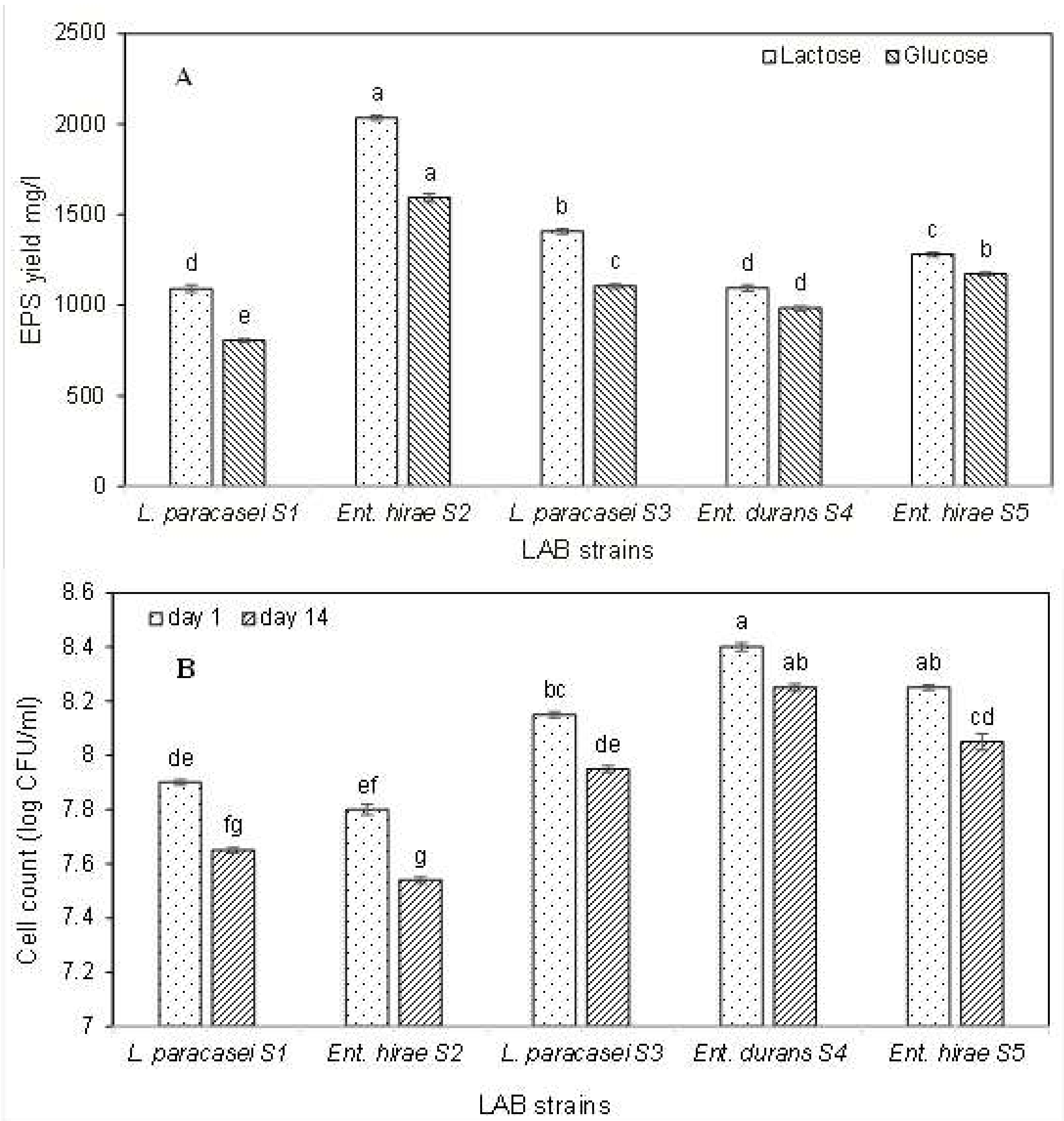


Fig. 2 Yield of exopolysaccharides-producing by isolated LAB strains (A) and changes in the cell viable counts of identified LAB strains during cold storage (B). Values marked with identical letters do not differ significantly

(2019) isolated three strains of *Enterococcus durans* from different artisanal dairy products and they found that all strains exhibited a high tolerance to pH 2.5 for 3 h.

Bile salts tolerance

Bile salts are considered toxic for bacterial cells because they disturb the cell membrane composition. Hence, the tolerance to bile salts is considered as a crucial property of the probiotic LAB that makes it grow, viability and existing its activity through gastrointestinal transit (Choi and Chang 2015). All identified strains exhibited well growth against the bile salts (sodium taurocholate) at level of 0.2 and 0.3% for 3 h except *Ent. hirae* S2 and S5. However, *Ent. durans* S4 showed a significant decrease in the viability after 3 h at 0.3% of bile salts (Table 2). In addition, all identified LAB strains appeared tolerance to bile salts at level of 0.5% for 2 h except *Ent. durans* S4. Our study illustrated the viability of *L. paracasei* S3 and *Ent. hirae* S5 in bile salts medium even at a high level (0.5%) for 3 h was acceptable. The tolerance of bile salt differs significantly due to specific nature of LAB and the resistance mechanism to bile salt concentration is species and strain dependent (Aarti et al. 2017). A similar trend has been reported by Zhao et al. (2021) who found a high tolerance of *L. plantarum*, *L. paracasei*, and *L. fermentum* strains to bile salts. Also, Nami et al. (2019) reported that three strains of *Ent. durans* showed a high tolerance to bile salts at 0.3% for 4 h.

Table 2: Bile salts tolerance ($OD_{620\ nm}$) of isolated strains at concentrations of 0.2%, 0.3% and 0.5%

Strains	Bile salts 0.2%		
	Zero	120 min	180 min
L. paracasei S1	0.405±0.001^{a}	0.392±0.012^{a}	0.391±0.012^{a}
Ent. hirae S2	0.409±0.011^{a}	0.405±0.008^{a}	0.398±0.022^{a}
L. paracasei S3	0.315±0.003^{b}	0.295±0.013^{b}	0.294±0.005^{b}
Ent. durans S4	0.246±0.009^{c}	0.245±0.005^{c}	0.238±0.005^{c}
Ent. hirae S5	0.307±0.012^{b}	0.305±0.012^{b}	0.302±0.012^{b}
Strains	Bile salts 0.3%		
	Zero	120 min	180 min
L. paracasei S1	0.241±0.011^{e}	0.236±0.002^{ef}	0.233±0.002^{ef}
Ent. hirae S2	0.306±0.02^{b}	0.295±0.012^{bc}	0.281±0.012^{cd}
L. paracasei S3	0.362±0.013^{a}	0.353±0.005^{a}	0.342±0.008^{a}
Ent. durans S4	0.226±0.021^{ef}	0.214±0.002^{fg}	0.193±0.01^{g}
Ent. hirae S5	0.298±0.001^{bc}	0.291±0.001^{bc}	0.264±0.01^{d}
Strains	Bile salts 0.5%		
	Zero	120 min	180 min
L. paracasei S1	0.292±0.011^{b}	0.272±0.011^{bcd}	0.258±0.012^{cde}
Ent. hirae S2	0.251±0.009^{de}	0.239±0.02^{ef}	0.212±0.001^{g}
L. paracasei S3	0.277±0.011^{bc}	0.268±0.01^{cd}	0.259±0.02^{cde}
Ent. durans S4	0.324±0.012^{a}	0.264±0.007^{cd}	0.221±0.001^{fg}
Ent. hirae S5	0.272±0.002^{bcd}	0.261±0.003^{cde}	0.251±0.01^{de}

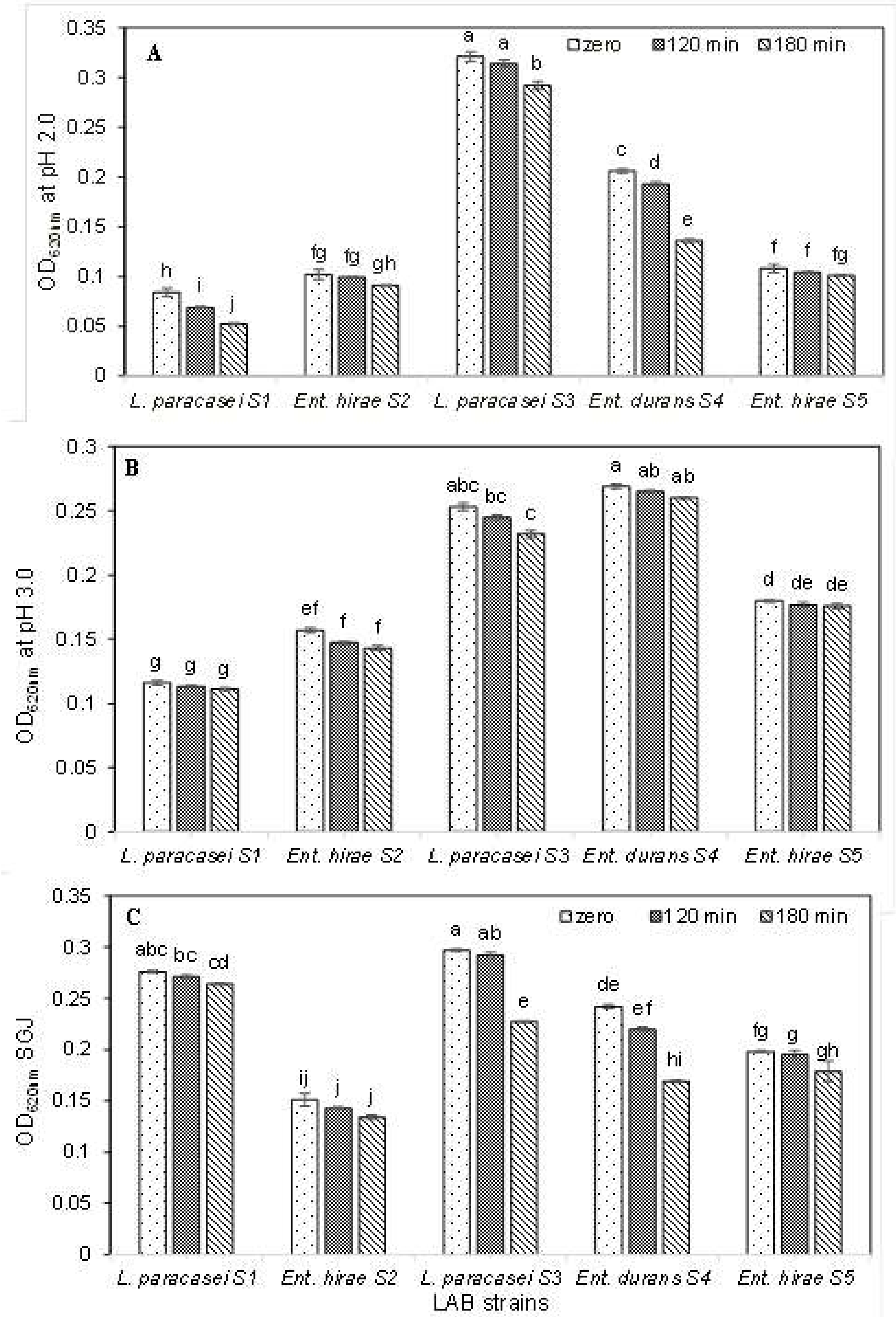
Values of each level marked with identical letters do not differ significantly

Table 3: Antibiotic resistance profile (Inhibition zone diameter, mM) of isolated bacterial strains

Strains	Antibiotics					
	Chloramphenicol	Tetracycline	Vancomycin	Penicillin	Lincomycin	Erythromycin
L. paracasei S1	17.0±1.0^{c}	21.0±1.5^{c}	ND	16.0±1.0^{b}	16.0±0.5^{c}	15.0±1.0^{c}
Ent. hirae S2	15.0±1.0^{d}	21.0±1.0^{c}	ND	15.0±0.5^{b}	16.0±1.0^{c}	20.0±1.5^{b}
L. paracasei S3	22.0±2.0^{b}	28.0±2.0^{a}	ND	22.0±1.0^{a}	20.0±1.5^{b}	25.0±2.0^{a}
Ent. durans S4	24.0±1.0^{a}	25.0±1.5^{b}	20.0±1.5^{a}	ND	22.0±2.0^{a}	23.0±2.0^{a}
Ent. hirae S5	12.0±0.5^{e}	17.0±1.0^{d}	20.0±2.0^{a}	12.0±0.5^{c}	10.0±0.5^{d}	12.0±0.5^{d}

Values marked with identical letters do not differ significantly.
ND: Not detected.

Fig. 3 Acid tolerance at pH 2 (A), pH 3.0 (B) and tolerance to simulated gastric juice (C) of identified LAB strains. Values marked with identical letters do not differ significantly.



Antibiotic resistance

The susceptibility of LAB strains to antibiotics is a remarkable feature assessing the probiotic bacteria safety where they may be utilized for antibiotic resistance genes of host. In this study, two groups of antibiotics were investigated, and these antibiotics were categorized by their mode of action to: cell wall creation restraints (penicillin and vancomycin) and protein formation restraints including chloramphenicol, lincomycin, tetracycline and erythromycin (Kapoor et al. 2017). The data in Table 3 illustrate that the studied *L. paracasei* strains and *Ent. hirae* S2 were resistance to vancomycin whereas they were sensitive to chloramphenicol, penicillin, tetracycline, lincomycin and erythromycin. *Ent. durans* S4 strain showed sensitive following the cut-off points given by European Food Safety Authority “EFSA” to chloramphenicol (16 mg/L), tetracycline (4 mg/L), vancomycin (4 mg/L), and erythromycin (4 mg/L) whereas it was resistance to penicillin. However, *Ent. hirae* S5 exhibited sensitivity to all antibiotics under study (Table 3). The resistance noted versus some antibiotics examined proposes that these bacteria would not be influenced by remedies and might aid preserve the normal equilibrium of intestinal microflora during antibiotic therapies. Depending on our results, it was proposed that antibiotic resistance and sensitivity of LAB varies with diverse species too (Solieri et al. 2014). Egervarn et al. (2009) and Shahali et al. (2023) reported that several *Lactobacillus* strains is susceptible to the cell wall-objective penicillin, and it is ordinarily sensitive to antibiotics that block protein formation including chloramphenicol, lincomycin and tetracycline. The impedance of vancomycin is quite recorded in Lactobacilli strains that are due to the formation of adjusted cell wall peptidoglycan precursors that finish in a depsipeptide D-alanine-D-lactate rather than the dipeptide D-alanine-D-alanine, the aim for vancomycin activity (Stogios and Savchenko 2020). Moreover, Anisimova and Yarullina (2019) reported that the glycopeptide impedance in *Lactobacillus* strains is not of the transmissible kind.

Simulated gastric juice tolerance

The impact of simulated gastric juice on the activity of identified LAB isolates is presented in Fig. 3C. The incubation of the five identified strains in the simulated gastric juice (pH 2.0) exhibited that *L. paracasei* S1, *Ent. hirae* S2 and *Ent. hirae* S5 had properly good growth and they tolerated for 3 h with survival rates in range 88.74-95.65%. On the other hand, *L. paracasei* S3 and *Ent. durans* S4 showed well tolerance to simulated gastric juice for 2 h and the survival rates were 98.32 and 90.91%, respectively. Several scientific research have notified that *Lactobacillus* and *Enterococcus* isolated from fermented food products are tolerant to gastric and intestinal juice circumstances (Abosereh et al. 2016; Nami et al. 2019). Also, El-Dieb et al. (2010) found that *L. casei* 01 could survive well under simulated gastrointestinal tract conditions.

The viability of identified LAB during cold storage

Since the survival of probiotic bacteria is deemed a crucial parameter for developing probiotic fermented milk, it is necessary to evaluate the viability of identified LAB at the end of skim milk fermentation and after two weeks of cold storage. The results in Fig. 2B showed that cold storage exhibited a negative impact on the viability of all identified strains except *Ent. durans* S4. However, the counts of all identified strains were above the lowest recommended therapeutic standard of probiotics (6 log CFU/ml) (Hadjimbei et al. 2022) at the end of storage period. Meybodi et al. (2020) reported that dissolved oxygen and post-acidification may have a negative impact on the viability of probiotic bacteria in fermented milk products. Also, Abd El-Fattah et al. (2018) found that as the titratable acidity of yogurt increases during storage period, the viable count of probiotics decreases, and it is probably due to acid injury. In this regard, Sah et al. (2015) found that the viability of *L. paracasei* decreased after 14 days of cold storage of probiotic yogurt.

Conclusion

In the current study, 40 LAB strains were isolated from 23 conventional Egyptian milk products, whilst five strains were chosen depending on the highly yield of EPS. These strains were identified as *Lacticaseibacillus* and *Enterococcus* genus. The highest EPS yield was observed for *Enterococcus hirae* S2 and *L. paracasei* S3. The five identified strains exhibited significant variations in tolerance to low pH, bile salts, simulated gastric juice, and antibiotics. The viable counts of all identified strains declined significantly after 14 days of cold storage except *Ent. durans* S4 but the counts were kept above 6 log CFU/ml. The present study proposed that conventional Egyptian dairy products can act as origins for the isolation and identification of probiotic LAB. Furthermore, our findings revealed that five identified LAB strains had considerable potential to be utilized in fermented food products. Moreover, using the genus of *Enterococcus* in cheese can affect the health of consumers in appropriate manners, thus making them a significant source of probiotic bacteria. More scientific studies are required to assess the health impacts of identified isolates in fermented food.

Acknowledgements

Authors thank Cairo University Research Park (CURP), Faculty of Agriculture, Cairo University for their help in the analysis. Authors are grateful to Dr. Samia Mahmoud El-Dieb, Professor of Dairy Science, Faculty of Agriculture, Cairo University for her assistance in the revision of article. Also, authors are grateful to Dr. Ashwak Abdel- Moneim Hassan, Lecturer of Dairy Sciences, Faculty of Agriculture, Cairo University for her help.

Funding No funding was received for carrying out this study.

Conflict of interests All authors declare no competing interests.

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