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The protective potential of selected lactic acid bacteria against the most common contaminants in various types of cheese in Egypt

Mamdouh S. Al-Gamal [a], Gamal A. Ibrahim [b], Osama M. Sharaf [b], Ahmed A. Radwan [a], Nadia M. Dabiza [b], Ahmed M. Youssef [c], Mohamed F. El-ssayad [b,*]

[a] *Botany and Microbiology Dept., Faculty of Science (Boys), Al-Azhar University, Cairo, Egypt*

[b] *Dairy Science Dept., (Dairy Microbiol. Lab.), National Research Centre, 33 El-Bohouth St. (Former El-Tahrir St.) Dokki, Giza, Egypt*

[c] *Packing and Packaging Materials Dept., National Research Centre, 33 El-Bohouth St. (Former El-Tahrir St.) Dokki, Giza, Egypt*

[*] Corresponding author.
E-mail address: sayad.nrc2012@yahoo.com (M.F. El-ssayad).

Abstract

Dairy products, especially cheeses have a great nutritional value and a high consumption level around the world. Considering a widespread consumption of cheeses, there is a growing concern regarding safety and microbiological quality. The current study was designed to conduct a recent evaluation of cheeses microbiological quality. Sixty cheese samples from retailing Egyptian markets were analyzed on different selective microbiological media and 64 bacteria, 35 yeasts and 8 molds were isolated. Out of 60 samples; 26.6% were contaminated with *Escherichia coli*, 73.3% with *Staphylococcus scuiri*, 3.33% with *Bacillus cereus*, 1.66% with *Salmonella enterica*, and 1.66% with *Pseudomonas aeruginosa*. The presence of such microorganisms in cheeses referred to the wrong management in cheese manufacturing. These organisms are significant from public health view as they have been associated with the base of human

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food poisoning. Promising antagonistic behavior was observed using the tested lactic acid bacteria (LAB) either single or in combinations toward the undesired isolates. *Lactobacillus helveticus* CNRZ 32 (*Lb. helveticus*) was the most potent culture; recording ≥95% reduction in undesired microbial counts.

Keywords: Food science, Microbiology, Food technology, Biotechnology, Food safety

1. Introduction

Milk and dairy products represent excellent growth media for many opportunistic food spoilage and disease-causing microorganisms. In industrial countries, *Staphylococcus aureus* (*Staph. aureus*), *Salmonella* spp. (*Sal.* spp.), *Listeria monocytogenes* and *Escherichia coli* (*E. coli*) are the most commonly detected pathogens associated with milk and dairy products. Also, these organisms resemble the main microbiological risks linked to raw milk, post-processing contaminated cheese or improperly treated milk (Cancino-Padilla et al., 2017).

Cheese is one of the most common foods over the world, and according to the International Dairy Foods Association report (IDFA, 2010), cheese is the major manufactured dairy product, with the developing importance of the dairy industry.

Outbreaks due to consumption of cheese contaminated with pathogenic bacteria and/or their toxins have the most importance of public health and economic consequences. Losses due to outbreaks include medications, charges, increased production wastes, loss of business, recall and damage of products, and investigation of the outbreaks (Röhr et al., 2005).

Salmonellosis; illness caused by *Salmonella spp.* is one of the most important disease problems for human and animal health, that causes worldwide human and animal sickness and sometimes death (Pal et al., 2015).

In addition, several strains of *Bacillus cereus* (*B. cereus*) are well known as toxin producers causing food poisoning (Tewari and Abdullah, 2015).

A variety of psychrotrophic bacterial species that primarily represented by *Pseudomonas spp.* (*Ps. spp.*) in the cold chain of raw milk can produce heat-stable proteases. These enzymes may spoil pasteurized milk (72−75 °C/15−20 s) even Ultra High Temperature-treated milk (130−150 °C/2−4 s) and dairy products (Marchand et al., 2009). Besides exhibiting spoilage features, some psychrotrophs possess an opportunistic pathogenicity. Also, these species are inherently toxin producers and/or resistant to antibiotics. Regarding quality, this bacterial group has

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become a key problem for today's dairy industry as causing of spoilage and significant economic sufferers (Samaržija et al., 2012).

Molds and yeasts are important contaminants of dairy product as a favorable niche for their growth. They are causes of visible or non-visible defects, such as off-odor and off-flavor, leading to cheeses waste and loss (Garnier et al., 2017).

Recently, consumers have an increasing awareness of their health risks caused by the utilization of chemical preservatives. So, this is a growing need in the dairy industry to extend product shelf-life and prevent spoilage by natural preservatives and/or new methods of conservation (Silva et al., 2018).

The group of lactic acid bacteria has a long history of safe application in dairy and other industries. Since they possess no health risks, bacteriocins and other bioactive compounds, that excreted by LAB strains, are a great substitute for chemical preservatives in dairy products. Among the most protective and health promoting LAB cultures; are *Lb. helveticus*, *Lb. plantarum*, *Lb. reuteri*, *Lb. rhamnosus*, *Lactococcus lactis*, *Streptococcus thermophilus* (Ranadheera et al., 2017) and *Enterococcus faecium* (Cavallini et al., 2011).

The main objective of this study was to recently evaluate and estimate the incidence of different foodborne pathogens and/or food spoilage microorganisms in some Egyptian dairy markets. Secondly, identification of a model set of the total isolates by sequencing of PCR-amplified rDNA gene fragments. Screening of different LAB for availability as protective cultures to inhibit the growth of foodborne isolates; bacteria, yeasts, and fungi was the last objective.

2. Materials and methods

2.1. Materials

Nutrient agar was provided from Panreac Quimica, Spain; Pseudomonas agar and MacConkey broth were provided from LAB m, UK; MRS agar was purchased from SRL, India; M17 agar was supplemented by CONDA, Spain; Pseudomonas C-N supplement, Baird-Parker agar, *Bacillus cereus* agar, Reinforced Clostridial agar and their supplements were purchased from Oxoid, England; EMB agar was obtained from HIMEDIA, India; and Malt extract agar was imported from Biolife, Italy.

2.2. Sample collection

Sixty cheese samples representing four different types; Talaga, Ras, Domiati, and Feta cheeses were collected from retailing markets in three different Egyptian Governorates; Cairo, Giza, and Menofia.

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2.3. Lactic acid bacteria strains

Seven LAB cultures were obtained from the collection of dairy Microbiological Lab., National Research Centre, Dokki, Giza, Egypt. Table 1 shows sources and references of these strains.

2.4. Microbiological examination

2.4.1. Recent evaluation of the microbiological quality

Isolation of pathogenic bacteria, molds and yeasts from the four cheese types were preceded under full aseptic conditions.

Ten grams of each sample were homogenized in 90 ml of 2% (w/v) sodium citrate solution. And serial 10 fold dilutions were prepared from this homogenate using 0.89 % (w/v) physiological saline solution.

As described by El-Hadedy and Abu El-Nour (2012), Bromo-Cresol Purple MaConkey broth tubes (LABm) were used for the total Coliforms counting. Fecal Coliforms were detected on EMB Agar.

Table 1. Protective cultures of lactic acid bacteria included in the study.

MRS agar-cultivated cultures	**Origin (Sources or reference)**
Lactobacillus plantarum DSA 20174	Collection of dairy Microbiological Lab., (supplemented with Cairo MIRCEN, Faculty of Agriculture, Ain Shams University. Egypt)
Lactobacillus helveticus CNRZ 32	Collection of dairy Microbiological Lab., (supplemented with Centre National de Recherche Zootechnique, Jouy-en-Josas, France)
Lactobacillus reuteri NRRL B-14171	Collection of dairy Microbiological Lab., (obtained from the Northern Regional Research Laboratory, Illinois, USA)
Lactobacillus rhamnosus NRRL B-442	Collection of dairy Microbiological Lab., (obtained from the Northern Regional Research Laboratory, Illinois, USA)
M17 agar-cultivated cultures	
Streptococcus thermophilus CH-1	Collection of dairy Microbiological Lab., (obtained from Chr. Hansen Lab., Denmark)
Enterococcus faecium FSD	Provided by Dr. Mohammed G. Shehata, City of Scientific Research and Technological Application, Egypt. He isolated it from a homemade Egyptian karish cheese (Deraz et al., 2015)
Lactococcus lactis subsp. *lactis*	Provided by Dr. Mohammed G. Shehata, City of Scientific Research and Technological Application, Egypt. He isolated from an Egyptian Rayeb Milk (Shehata et al., 2016)

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According to Ollis et al. (1995), Baird-Parker agar medium was used for Staphylococci counting. The presence of *Staphylococcus aureus* was confirmed by the coagulase test (Baird-Parker, 1962).

For detection of *Salmonella* and *Shigella*, 25 g of sample were added to 225 ml of sterile buffered peptone water for pre enrichment. After incubation at 37 °C/24 h, 10 ml of growth suspension, were transferred to 90 ml boiling-sterilized Selenite broth. After incubation, XLD plates were streaked from Selenite broth (Taylor and Harris, 1965).

Molds and yeasts were counted on malt extract agar. Plates were incubated for 72 h at 30 °C, and then analyzed for fungal population (log CFU/gram).

2.5. Identification of the foodborne isolates

2.5.1. Morphological and biochemical characterization

For detection of *Shigella* and *Salmonella*; pink colonies with negative or positive H_2S were respectively detected on XLD agar after 24 h/37 °C. *Salmonella*; the positive H_2S (black centre) colonies were then streaked on urease slants to confirm the negative reaction.

E. coli was distinguished by purple colonies with a green metallic sheen on EMB agar. *Pseudomonas aeruginosa* developed bluish green pigmented colonies on Pseudomonas agar.

For the last 4 organisms, colonies showed positive in the catalase test (Staphylex, Oxoid) and negative gram reaction, had been confirmed.

Bacillus cereus developed peacock blue colonies with a precipitate on a *Bacillus cereus* agar plate. Characteristic colonies showing a positive gram reaction, positive catalase test, and spore forming rod-shaped cells were confirmed as *Bacillus cereus*.

Baird−Parker agar was used to isolate Staphylococci where representative gram-positive clustered cocci, typical black appearance colonies and surrounded by clear zone were picked up, and tested for catalase. Colonies showed egg yolk lysis and positive catalase were confirmed as *Staph. aureus.*

2.5.2. Molecular identification

2.5.2.1. DNA extraction

A single microbial colony from each selective agar plate was aseptically sent to the GIS Research Centre (6th October City, Egypt) for DNA extraction, rRNA PCR amplification and sequencing. DNA was extracted, amplified and purified using

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Quick-DNA Miniprep Plus Kits, ZYMO RESEARCH CORP., Irvine, California, United States, according to manufacturer protocol for solid tissues.

2.5.2.2. PCR conditions and primers

Reactions were performed using Delbes et al. (2007) method with slight modifications as follows: in a final volume of 50 μl. The reaction mixture contained 8 μl template, 25 μl MyTaq Red Mix (2x), and 1 μl of each primer (conc. 20 μmol/l). The amplification program was denaturation; 1−5 min, 95 °C (1 cycle), then 30−36 cycles of denaturation (40−120 Sec, 95 °C), annealing (90−120 Sec, 55 °C), while annealing temperature was 60 °C in case of bacteria; and extension (5−7 min, 72 °C). Ten μl of PCR-amplified product was analyzed by electrophoresis on 0.9 % ethidium bromide-stained agarose gel where the DNA ladder (0.3 mg/L) or bacterial suspension (2 μl) was used as a template. Table 2 contains specific primers with expected amplicon sizes for bacteria, fungi and yeast:

Then the resulted sequences were aligned with the National Center for Biotechnology Information (NCIB) database using Basic Local Alignment Search Tool (BLAST) (https://blast.ncbi.nlm.nih.gov/Blast.cgi), to choose the highest matching (Brindha and Mathew, 2012).

2.6. Antimicrobial assays

2.6.1. Qualitative screening of LAB against spoilage and pathogenic isolates

The assays were performed using the agar overlay technique as described by Shokryazdan et al. (2014) with slight modifications. Overnight cultures of lactic acid bacterial strains that listed in Table 1a were separately mixed with MRS or M17 agar ($\approx 10^5$ CFU/100 μl). One hundred μl from each strain were pipetted to fill wells that previously made in agar plates, using a micropipette, and the inoculated plates were incubated at 37 °C for 48 h.

Table 2. Primers used for PCR, with the expected amplicon size.

Isolate type	Primers	Amplicon size
Bacteria (16S rDNA)	F 5′-CAGGCCTAACACATGCAAGTC–3′ R 5′-CGGCGGWGTGTACAAGGC -3′	1500 bp
Mold (18S rDNA)	F 5′ CTT GGT CAT TTA GAG GAA GTA A 3′ R 5′ TCC TCC GCT TAT TGA TAT GC 3′	750 bp
Yeast (26S rDNA)	F 5′-GCATATCAATAAGCGGAGGA AAAG-3′ R 5′- GGTCCGTGTTTCAAGACGG-3′	700 bp

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The agar plates containing the growth of lactic acid bacteria in spot forms (5 mm diameter) were then overlaid with soft nutrient agar (0.8% agar) pre-mixed with 10^8 CFU of the indicator stains, and incubated, after the solidification of the overlaid agar layer, at 37 °C for 24 h. The zone diameter of inhibition values obtained were measured and interpreted. The antifungal activity of LAB was investigated with an overlay assay using the modified method of (Magnusson and Schnürer, 2001; Lind et al., 2005). LAB cultures on agar were poured in 2 cm × 1 cm grooves on MRS agar plates. Ten milliliter of soft (0.8%) malt extract agar containing 1 ml of inoculum of mold spore or yeast suspension was then poured onto the agar plates and incubated at 30 °C. After 48 h, the zone of inhibition was measured. The degree of inhibition was calculated as the area of inhibited growth.

2.6.2. Co-culturing of foodborne isolates with LAB (quantitative interaction)

As described by Ammor et al. (2006), the most potent antimicrobial LAB was co-cultivated with the previously isolated foodborne microbial cultures separately in dual species planktonic cultures. Log CFU was recorded for the foodborne isolates after 24 h in comparison with the control.

2.7. Statistical analysis

Statistical significance was determined using Statistica Version 9 (State Soft, Tulsa, Okla., USA). The means were determined by analysis of variance test (ANOVA, two way analysis) ($p < 0.05$). Fisher's LSD (Least Significant Difference) Method ($\alpha = 0.05$) was applied to compare significant differences between treatments (Williams and Abdi, 2010).

3. Results and discussion

3.1. Microbiological quality evaluation of cheese

The results of the recent microbiological evaluation conducted on 60 cheese samples that categorized under main four cheese types were collected from different markets from three different Egyptian governorates; Cairo, Giza and Menofia. The results are presented in Tables 3, 4, 5, 6, and 7.

It is well-known that the effectiveness of the pasteurization process and the resulting quality of cheese and other dairy products directly reflect the microbiological quality of the raw milk (Nörnberg et al., 2010). Table 3 illustrates the microbial counts (log CFU/g) of the dominant foodborne pathogens in the tested cheeses. Feta cheese contained the highest total viable count (7 ± 4.28 log CFU/g), and the predominant factor was Psychrotrophic bacteria whose count reached (3.6 log CFU/g), while the

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Table 3. Microbiological analyses of different cheese types.

Tested microorganisms	Microbial counts (Log CFU/g)			
	Talaga (n = 15)	Ras (n = 15)	Domiati (n = 15)	Feta (n = 15)
Coliform group	2.5 ± 0.80	2.1 ± 1.41	0.3 ± 0.47	Nil
Staphylococci	5.8 ± 1.77	6.5 ± 1.07	3.6 ± 1.86	1.6 ± 1.06
Aerobic spores	3.4 ± 0.72	3.7 ± 1.06	2.4 ± 0.83	Nil
Anaerobic spores	Not tested	Not tested	Not tested	Nil
Psychrotrophic bacteria	1.8 ± 0.49	Nil	Nil	3.6 ± 2.92
Molds	1.1 ± 0.73	2.4 ± 1.69	2 ± 1.43	Nil
Yeasts	2.05 ± 1.44	4.5 ± 1.96	4.2 ± 2.48	Nil
Total viable count	6.8 ± 2.06	7.3 ± 0.85	6.1 ± 1.59	7 ± 4.28

Data expressed as mean ± standard error, Nil: No growth detected.

coliform group were not being detected. This may reflect the improper heat treatment of high microbial-loaded raw milk in some factories, the presence of biofilms in production lines and/or weak hygienic system as reported by Marchand et al. (2012). Lower than feta cheese, Talaga cheese whose total viable count reached 6.8 ± 2.06 log CFU/g, and Staphylococcal count achieved 5.8 ± 1.77 log CFU/g, Total coliforms count 2.5 ± 0.8 log CFU/g, molds and yeasts 1.1 ± 0.73 and 2.05 ± 1.44 log CFU/g respectively. Domiati and Ras types were distinguished by their high Staphylococcal contents (3.6 ± 1.86 and 6.5 ± 1.07 log CFU/g, respectively), while Ras cheese had the highest coliforms load that reached 4.3 log CFU/g in some samples. Domiati and Ras samples included the largest fungal loads (2 ± 1.43 and 2.4 ± 1.69 log CFU/g for fungi and 4.2 ± 2.48 and 4.5 ± 1.96 log CFU/g for yeasts, respectively). According to Kean et al. (2017), there was a reported relationship between the yeast count and *Staphylococci* existence in food samples. It was thought that some yeast can structurally and nutritionally support the existence (adherence and colonization) of *Staphylococcus sp.* if the both exist in the same environment. According to that reported by Johler et al. (2015), *Staph. aureus* was determined as a causative agent of a food poisoning outbreak On October 1, 2014. Within the first 7 hours after the meal, all persons consumed a soft cheese produced from

Table 4. Incidence of coliforms in the examined cheese samples.

Cheese type	Positive samples	%
Talaga	13/15	87
Domiati	12/15	80
Ras	2/15	13.3
Feta	0/15	0
All	**27/60**	**45**

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raw cow milk fell ill. Investigations revealed that the soft cheese exhibited low levels of staphylococcal enterotoxin A and high levels of enterotoxin D. It was considered that temperature abuse above 10 °C and the reduced contribution of inactive starter culture during fermentation are key factors in staphylococcal-based dairy outbreaks (Cretenet et al., 2011).

Table 4 records the percentage of coliforms contamination of different cheeses. The total coliforms count was increasingly noted in the direction from Feta to Talaga cheeses. Coliforms presence indicates the insufficient processing and post-processing stages, and failure of the Good Manufacturing Practices (GMP) or hygienic control either during manufacture or distribution of these products. Focusing on cheese samples contaminated with coliforms, Trmčić et al. (2016) results represented 50% of these study findings. In addition, this suggested explanation of bad quality was supported by that mentioned by Hervert et al. (2017) and his team. They reported that the *Enterobacteriaceae* and total gram-negative groups more accurately reflect the hygienic status of the processed milk, processing, and storage environments.

The importance of these bacteria is that they have become components of the microbiological evaluating programs of both industry and guiding organizations. Principally, they may be considered as signs of safety or quality of dairy products. Presence of these signs in food products proposes the presence of conditions associated or suitable for other undetectable pathogens. In addition, they have a history of constant ecological association with the pathogen in the environment where contamination initiates (Price and Wildeboer, 2017). So these quality signs assess important conditions for product manufacturer or customer acceptability (Tortorello, 2003).

Table 5 represents the numbers and percentages of different contaminants in the examined cheese types. About 26.6% of the total samples were positive for *E. coli*; the most indicator organism of fecal contamination. Some serotypes of this organism have a high virulence e.g. *E. coli* O157:H7; a Shiga toxin-producing *Escherichia coli* (STEC) which can cause disease at a dose of 5−50 cells (Farrokh et al., 2013).

The main source of STEC is ruminants that contaminate milk over subclinical mastitis or fecal ways, and the bacteria can stay in milking apparatus.

3.2. Identification of the isolated pathogenic and spoilage microorganisms

Table 6 summarizes the morphological and biochemical grouping of bacterial isolates. Depending on the most important biochemical and morphological tests, out of the 64 bacterial isolates 16 were identified as *E. coli*, one *Salmonella sp.*, one

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Table 5. Distribution of the bacterial contaminants in different cheeses types.

Pathogen	Positive samples by Cheese type				Total positive samples	Positive samples %
	Talaga	Domiati	Ras	Feta		
E. coli	4/15	2/15	10/15	0/15	16/60	**26.6**
Sal. enterica	0/15	1/15	0/15	0/15	1/60	**1.66**
Ps. aeruginosa	0/15	1/15	0/15	0/15	1/60	**1.66**
Staph. scuiri	13/15	15/15	13/15	3/15	44/60	**73.3**
Bacillus cereus	0/15	2/15	0/15	0/15	2/60	**3.33**

Pseudomonas aeruginosa, two *Bacillus cereus* and 44 were coagulase negative *Staphylococcus sp*.

Fig. 1 shows gel electrophoresis of the purified PCR products of representative isolates. Bands from 11 to 15 represented the bacterial 16S rDNA at 1500 bp., while bands 16 and 50 represented the fungus 18S rDNA and the yeast 26S rDNA at 600 and 699 bp. respectively. These PCR products were next subjected to nucleotide sequencing and then compared with the available sequences in the NCBI GenBank database using BLAST; one of the most greatly used sequence analysis tools available in the public domain. Table 7 represents homology % of the highest matched strains with their accession numbers.

There is now a wide choice of BLAST systems that can be used to search much different sequence databases and finds regions of matching between biological sequences (McGinnis and Madden, 2004). The obtained sequence matching for the isolates were very reliable ($\geq$96% similarity with the database).

According to Table 7, the alignment showed that the representative isolates had the highest identity with *Escherichia coli* strain E11 (accession number KY780346.1),

Table 6. Morphological and biochemical grouping of bacterial isolates.

Isolation media	Isolates number	Microscope	Gram staining	Catalase	Sporulation	others
EMB agar	**16**	Short rods	Negative	Positive	Negative	H_2S (−)
XLD agar	**1**	Short rods	Negative	Positive	Negative	H_2S (+) Urease (−)
Pseudomonas agar	**1**	Rods	Negative	Positive	Negative	Blue green pigment (+)
Bacillus cereus agar	**2**	Rods	Positive	Positive	Positive	Peacock blue colonies
Baird Parker agar	**44**	Cocci in clusters	Positive	Positive	Negative	Coagulase (−)
Total isolates	**64**					

−: negative, +: positive.

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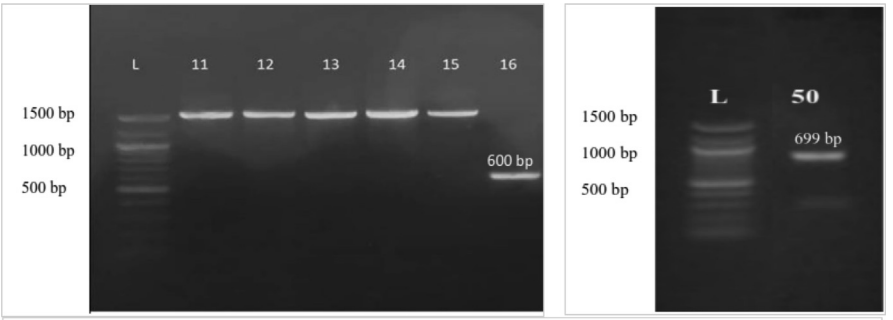

Fig. 1. Gel images of rDNA PCR products for the representative cheese contaminants.

Salmonella enterica strain SA19992307 (accession number CP030207.1), *Pseudomonas aeruginosa* strain Kasamber5 (accession number KY549641.1), *Bacillus cereus* strain 151007-R3-K09-40-27F (accession number KY820914.1), and *Staphylococcus sciuri* strain 2-6 (accession number MH491952.1). Ruaro et al. (2013) identified different coagulase-negative staphylococci from Italian raw milk and cheeses; *Staphylococcus sciuri* was among the recovered species and represented about 10% of the total community.

Mold and yeast representative isolates had the highest identity with *Penicillium chrysogenum* (*P. chrysogenum*) strain J127 (KF572447.1) and *Candida parapsilosis* (*C. parapsilosis*) strain F2-17 (KP852497.1) respectively.

3.3. Qualitative screening of LAB for antimicrobial activity against isolated pathogens

Table 8 presents antagonistic effect of different LAB cells against the representative microbial isolates. All the applied LAB strains showed inhibitory effects against all indictor food spoilage and pathogenic microorganisms, except *Str. thermophilus* and *Ent. faecium* in the case of *Ps. aeruginosa*. Also, Fig. 2 clearly

Table 7. Homology percentages of isolates nucleotide sequences.

Code	Identification	Isolation media	Accession number	Homology %
11	*Escherichia coli*	EMB agar	KY780346.1	98 %
12	*Salmonella enterica*	XLD agar	CP030207.1	96 %
13	*Pseudomonas aeruginosa*	Pseudomonas agar	KY549641.1	98 %
14	*Bacillus cereus*	Bacillus cereus agar	KY820914.1	99 %
15	*Staphylococcus sciuri*	Baird-Parker agar	MH491952.1	100 %
16	*Penicillium chrysogenum*	Malt extract agar	KF572447.1	99 %
50	*Candida parapsilosis*	Malt extract agar	KP852497.1	99 %

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Table 8. Antimicrobial efficacy of pure LAB cells against representative contaminants.

LAB cultures	Pathogenic & spoilage strains						
	Staph. scuiri	*B. cereus*	*Sal. enterica*	*E. coli*	*Ps. aeruginosa*	*P. chrysogenum*	*C. parapsilosis*
Lb. plantarum	+++	++	+++	+++	+	+++	++
Lb. helveticus	+++	++	+++	+++	+	+++	++
Lb. rhamnosus	++	++	+	++	+	+	+
Lb. reuteri	++	++	+	++	++	+	+
Str. thermophiles	++	+	+	+	ND	+	+
Ent. faecium	+	+	+	+	ND	+	+
Lactococcus lactis	++	++	+	++	++	+	+

+: 5 mm, ++: 6−10 mm & +++: 10:15 mm and ND: non-detected inhibition.

showed model results of inhibition with different zone diameters confirming what presented in Table 8. As shown in Fig. 2, zones of inhibition are greater for bacterial isolates than fungus than yeast. The observed greatest inhibition in the case of *Lb. plantarum* and *Lb. helveticus* may be caused mainly by excessive acid production. Making this suggestion more acceptable is the weak response of yeast; *Candida parapsilosis* toward the inhibition. Many studies guaranteed these results explaining as those LAB strains can produce antimicrobial agents that − in different mechanisms-develop a strong inhibitory activity against many microorganisms, including pathogenic and spoilage ones. Metabolites such as organic acids (lactic and acetic acid), hydrogen peroxide, ethanol, diacetyl, acetaldehyde, acetoin, carbon dioxide, and bacteriocins (Šušković et al., 2010), are examples of antimicrobial agents produced by LAB. Organic acid produced by LAB resulted in low pH levels and activity of hydrogen peroxide (Ponce et al., 2008). Organic acids and other products exhibit antibacterial activity against various pathogenic Gram-positive and Gram-negative microorganisms (Maragkoudakis et al., 2009).

As data showed, most of LAB cultures possess antimicrobial activities in different degrees against all indicator isolates. The observed degree of inhibition seemed to be specific to the protective culture and the indicator stain. As mentioned above, it is clear that both *Lb. helveticus* and *Lb. plantarum* showed the strongest antagonism of 10−15 mm zone of inhibition against *Staph. scuiri, Sal. enterica, E. coli and P. chrysogenum*, while lower activity (6−10 mm) was shown against *B. cereus, Ps. aeruginosa* and *C. parapsilosis*. Mols et al. (2010) observed the same behavior in *B. cereus* stating that such behavior was due to the activation of some important enzymes that drive intracellular pH hemostasis. And Desriac et al. (2013) reported that the response of *B. cereus* to acid stress could be a general stress response, pH homeostasis, metabolic rearrangements, or secondary oxidative stress response. Also, *Ps.*

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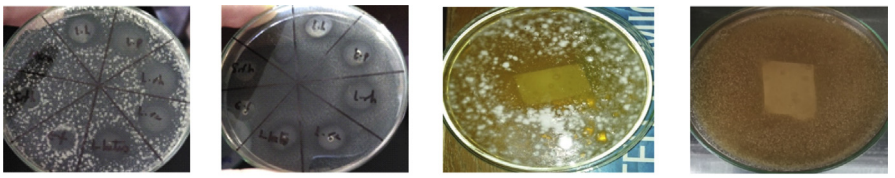

(a) *B. cereus* (b) *E. coli* (c) *P. chrysogenum* (d) *C. parapsilosis*

Fig. 2. Interaction between selected LAB and some cheese contaminants.

aeruginosa was reported by Slabbert (2013) to show intermediate tolerance toward low pH.

In Tables 9 and 10, the shown data represented the effect of different LAB culture combinations in enhancing the antimicrobial activity against the indicator isolates.

It was noted that the antifungal activity disappeared in all cases, while the antibacterial activity was stimulated, especially in case of *Lb. helveticus*-based combinations. This pattern appeared as there is a synergistic relationship drives the antimicrobial activity by *Lb. helveticus*-based combinations. Considering the antibacterial activity, some researchers reported that the S-layer −upon combination with bacteriocins-can act synergistically to inhibit the growth of both gram positive and gram negative bacteria (Prado-Acosta et al., 2010). Also, they suggested that the surface layer of lactobacillus membrane enables bacteriocin to cross the pathogen cell wall, while bacteriocin provides requirements for S-layer murein hydrolase activity.

3.4. Co-culturing of foodborne isolates with protective culture

Table 11 shows the role of *Lb. helveticus* in the reduction of microbial count (Log CFU/ml). As presented, after co-culturing of *Lb. helveticus* with different indicator strains, bacterial count (log CFU/ml) was reduced by $>$ 99.99% and fungal counts became undetected after 24 h, while the yeast count was reduced only by about 99.5% over the same incubation period. The observed behavior of *C. parapsilosis*

Table 9. Antimicrobial efficacy of LAB mixed cultures against representative contaminants.

Combination		Pathogenic & spoilage strains						
		Staph. scuiri	*B. cereus*	*Sal. enterica*	*E. coli*	*Ps. aeruginos*	*P. chrysogenum*	*C. parapsilosis*
Lb. p	*Lactococcus lactis*	+	++	++	+++	++	ND	ND
	Str. thermophilus	+	++	++	+++	ND	ND	ND
	Ent. faecium	+	++	++	+++	ND	ND	ND
Lb. h	*Lactococcus lactis*	+++	++	++	+++	+	ND	ND
	Str. thermophilus	+++	++	+++	+++	ND	ND	ND
	Ent. faecium	+++	++	+++	+++	ND	ND	ND

Lb.rh: *Lb. rhamnosus*, *Lb.re*: *Lb. reuteri*, +: 5 mm, ++: 6−10 mm & +++: 10:15 mm, and ND: non-detected inhibition.

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Table 10. Antimicrobial efficacy of LAB mixed cultures against representative contaminants.

Combination		Pathogenic & spoilage strains						
		Staph. scuiri	*B. cereus*	*Sal. enterica*	*E. coli*	*Ps. aeruginosa*	*P. chrysogenum*	*C. parapsilosis*
Lb. rh	*Lactococcus lactis*	++	+	+	++	++	ND	ND
	Str. thermophilus	++	+	+	++	ND	ND	ND
	Ent. faecium	++	+	+	++	ND	ND	ND
Lb. re	*Lactococcus lactis*	+	+	+	+	+	ND	ND
	Str. thermophilus	+	+	+	+	ND	ND	ND
	Ent. faecium	+	+	+	+	ND	ND	ND

Lb.rh: *Lb. rhamnosus*, *Lb.re*: *Lb. reuteri*, +: 5 mm, ++: 6−10 mm & +++: 10:15 mm, and ND: non-detected inhibition.

Table 11. Microbial count reduction of cheese contaminants by *Lb. helveticus*.

Cultures	Pathogenic & spoilage isolates count (log CFU/ml) after 24 h						
	Staph. scuiri	*B. cereus*	*Salm. enterica*	*E. coli*	*Ps. aeruginosa*	*P. chrysogenum*	*C. parapsilosis*
Lb. helveticus	2.8 ± 0.06^{A}	3.9 ± 0.06^{AB}	4 ± 0.10^{AB}	4.5 ± 0.06^{AB}	4 ± 0.15^{AB}	0^{A}	5.7 ± 0.12^{AB}
Control	10.6 ± 0.15^{B}	10.4 ± 0.25^{B}	9.4 ± 0.15^{C}	10.7 ± 0.06^{E}	9.1 ± 015^{C}	5.1 ± 0.06^{D}	8.1 ± 0.15^{C}

Data expressed as mean ± standard error, $^{A-E}$ Different letters in a same column mean significant difference at $P < 0.05$.

confirmed its weak response to different LAB cultures which was observed in Tables 8, 9, and 10. This suggestion ensured that the major inhibitory effect of LAB is due to organic acids; Furthermore, Mawgoud et al. (2016) confirmed this by achieving the highest yield of lactic acid from *Lactobacillus bulgaricus* at 37 °C.

The observed strong antagonism between *Lb. helveticus* and other undesired isolates is a clear evidence of its metabolic efficiency to synthesize different antimicrobial substances or a broad-spectrum antimicrobial compound (Oldak et al., 2017). Several studies used the live *Lb. helveticus* culture to attain a successful protection from enterohaemorrhagic *E. coli* (Jandu et al., 2009), and other *in vivo* studies recommended the utilization of *Lb. helveticus* culture to benefit its ability to prevent gastrointestinal infections, enhance protection against pathogens, modulate the host immune responses, and affect the composition of the intestinal microbiota (Taverniti and Guglielmetti, 2012).

4. Conclusion

The current study reported the microbiological status of different cheese types from local retailing markets of three Egyptian governorates. Sixty four isolates represented in five bacteria, one mold, and one yeast. The isolates were molecularly identified by rDNA gene fragments sequencing. Alignment with NCIB database sequences revealed that most of them had highest identities with potentially

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pathogenic and/or food spoilage strains. LAB cultures showed a promising antagonistic behavior toward these undesired isolates either as single or in combinations.

The most potent culture; *Lb. helveticus* was further applied in co-cultures against different isolates showing ≥95% reduction in undesired microbial counts. Further studies are recommended, for extraction, purification, identification and structure elucidation of the bioactive compounds that can contribute to the development of a novel bio preservation system.

Declarations

Author contribution statement

Mamdouh S. Al-Gamal, Osama M. Sharaf, Ahmed A. Radwan, Nadia M. Dabiza: Conceived and designed the experiments; Wrote the paper.

Gamal A. Ibrahim: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Ahmed M. Youssef: Contributed reagents, materials, analysis tools or data; Wrote the paper.

Mohamed F. El-ssayad: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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The authors declare no conflict of interest.

Additional information

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