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Positive Impact of Potential Probiotic *Lacticaseibacillus rhamnosus* Isolated From Local Dairy Products on Raising Microbiological Quality of White Soft Cheese

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TRADITIONAL dairy products in rural areas could be an excellent source of beneficial natural bacteria. Lactic acid bacteria (LAB) would be the right choice to represent these indigenous isolates with probiotic characteristics. Therefore, the purpose of this study was to improve nonstarter Egyptian white soft cheese by using probiotic indigenous strain isolated from the New Valley region of the Egyptian Western Desert. The results showed that the selected isolate, *Lacticaseibacillus rhamnosus* CH08, showed high tolerance to low pH, pepsin, bile salts and was non-hemolytic. *Lb. rhamnosus* CH08 also contributed to improving the taste and flavor of white soft cheese. In addition, their promising characteristics as prospective probiotics may cause amendment in the gastrointestinal microbiota. Furthermore, the strain did not contain any of the tested antibiotic resistant genes. This study confirms the diversity of microbiota in traditional dairy products, and the selected probiotic strain, *Lb. rhamnosus* CH08, succeeded in developing the quality of Egyptian white soft cheese" with synergistic health benefits as potential probiotic supplements.

Key word: Potentialprobiotic, Lactic acid bacteria, *Lacticaseibacillus rhamnosus*

Introduction

Lactic acid bacteria (LAB) are part of the human commensal microbiota that is mostly present in many fermented and unfermented foods (Adams et al., 1999). Although this cluster of bacteria varies in their shapes, needs, and metabolites, they are still generally recognized as safe (GRAS). Furthermore, it can play a significant role in the biotechnology and dairy industries through the fermentation process. The term probiotic is defined by FAO/WHO as "living microorganisms that, when administered in adequate amounts, confer a health benefit on the host." However, the main obstacle to probiotics is their ability to tolerate the harsh conditions of low pH and the presence of bile salts in the stomach and upper intestine, respectively that affect their growth and viability (Sanders, 2008). Moreover, it cannot ignore the safety assessment of probiotics before any application in food processing that guarantees consumer safety and improves the properties of functional probiotic products. However, the spreading of antibiotic resistance and virulence genes is still an obstacle worldwide due to the increasing capability of pathogens to develop multidrug resistance. LAB can also play an important role as a reservoir of transferable antibiotic resistance and virulence genes between animals and humans (Popović et al., 2018). Moreover, most of the reported clinical cases are related to *Enterococcus* spp. and *Lactobacillus* spp. strains. Infections associated with *Lactocobacillus* spp. are mainly concerned with *Lactocobacillus rhamnosus, Lactocobacillus lactis* subsp. *lactis* and *Lactocobacillus garvieae* (Rossi et al., 2019).

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Therefore, monitoring antibiotic resistance and virulence genes is critical for preventing the spread of these determinants within food chain. Raw milk and dairy products represent a rich source of essential nutrients and beneficial lactic acid bacteria (Ahansaz et al., 2023). Hence, traditional dairy products such as yoghurt, cheese, and other fermented milk in a rural area are quite a good choice for isolating indigenous LAB strains for probiotic properties. Tallaga cheese is a type of white soft cheese that is widely present in the Egyptian market due to its mild, pleasant, creamy taste, low salt content, high nutritional value and reasonable price. It can also be ripened and stored at a low temperature 5±1 °C to be ready for consumption within one month of storage (Abd El-Salam & Benkerroume, 2006; Hamad & Naser El-Deen, 2021). Indigenous LAB isolates can play the main role in ripening process, which enhances flavor intensity and improves quality of cheeses. However, it is impossible to obtain a universal strain among them that has all the proposed positive effects. Many of the LAB strains belonging to the genera *Lactobacillus* that are commonly defined as probiotics were frequently isolated from cheese (Ningtyas et al., 2019). Probiotics widely used in fermented food or as supplements include *Lacticaseibacillus rhamnosus*, *Lactocobacillus plantarum*, *Lactocobacillus brevis*, *Lactocobacillus paracasei*, *Streptococcus thermophilus*, and *Lactocobacillus acidophilus.* Therefore, the aim of this study was to isolate, screen, and identify indigenous strains of LAB from different sources in the Egyptian Western Desert for their potential probiotic and sensory properties to improve the microbiological quality of white soft cheese.

Materials and Methods

Sampling and isolation of LAB

A total of 40 samples of raw milk, yoghurt, Kareish and Domiati cheese were collected from villages and farms in the Southwestern part of Egypt at the New Valley governorate. Four regions of this governorate were defined; Kharga, Dakhla Farafra and Paris for collecting natural wild isolates. The samples were diluted in an appropriate volume (1/9 w/v) of 0.1% peptone water (Merck, Germany) to 1×10^{-4}. Out of 80 colonies, 35 isolates of Lactic acid bacteria were collected randomly from MRS agar plates under anaerobic conditions at 37 °C for 48 hr. The selected colonies regarded as LAB candidates were preliminarily identified by Gram staining, catalase assay and fermentation of lactose. All collected LAB isolates were maintained in 20% glycerol at -20 °C. Then, the stored frozen collection isolates were reactivated in MRS medium for further analysis.

Safety assessment and screening for probiotic properties of the LAB

Hemolytic activity

The hemolytic activity of the selected LAB isolate was determined according to Maragkoudakis et al. (2006). LAB strains were inoculated in MRS broth at 37°C for 24 hr. Followed by streaking the culture onto a blood agar base (Oxoid, UK) containing sheep blood 5% (v/v) and incubating at 37 °C for 24 hr. The plates were observed for the formation of any clear zones (β-hemolysis), greenish hemolytic zones (α hemolysis), or no such zones (γ-hemolysis) around the selected colony. LAB strains that showed no β-hemolysis or α-hemolysis were considered safe.

Fermentation in skim milk

Only LAB that showed γ-hemolysis activity were determined for their sensory characteristics. The isolates were inoculated with (1.5%, v/v) into 100 ml skim milk. Then, they were incubated at 37 °C for 36 hr in order to define the selected LAB strains that gave the accepted fermentation coupled with excellent sensory properties.

Resistance to low pH tolerance and pepsin

Bacterial isolates determined for low pH and pepsin resistance as reported by (Plessas et al. 2017). Briefly, the isolates inoculated in MRS broth at 37°C for 24 hr. The pelletwas collectedafter centrifugation at 10000 ×g at 4 °C for 5 min. Then, cell pellet suspended into 10 ml sterilized phosphate buffer solution (PBS) previously adjusted to pH 2.0 with 0.1 N HCl and incubated at 37 °C for 3 hr. Viable bacterial counts were determined by plating serial dilutions (with PBS, pH 7.2) on MRS agar medium after incubation at 37°C for 48 hr.

Survival rate (%) = log CFU mL^{-1} 3h / log CFU mL^{-1} under normal condition 0h × 100.

0h= at 0 time under normal condition
3h= after 3 hour under acidic condition

Pepsin resistance was also determined, the cells pellet were collected from overnight cultures by centrifugation as described above. After that the pellet washed twice in PBS and suspended in PBS solution at pH 2.0 containing pepsin 3 mg/

mL (Sigma Chemical Co) sterilized by filtering through 0.22 µm membrane sterilized. Viable bacterial counts were determined on MRS agar plates after incubation at 37 °C for 0 and 3 hr with pepsin.

Survival rate (%) =
log CFU mL^{-1} for 3hr / log CFU mL^{-1} for 0hr × 100

0h= at 0 time under normal condition.
3h= after 3 hour at pepsin solution.

Bile salt tolerance and Bile salt hydrolase activity

The acid-surviving isolates at pH 2 that can also resist pepsin were tested for tolerance to bile salts. Bile salt tolerance was determined by inoculating with 7 log CFU/mL^{-1} of LAB isolates in MRS broth medium supplemented with 2% (w/v) bile salts (Merck, Germany) and incubated at 37°C for 24 hr (Mourad & Nor-Elddine 2006). Bile tolerance isolates were detected by comparing the viable LAB counts of the treatment with the control after incubation on MRS agar at 37 °C for 48 hr. The survival rate percentage was evaluated by counting the cell numbers for both on MRS agar.

Survival rate (%) =log CFU/mL^{-1} for 3hr / log CFU/mL^{-1} for 0h × 100

0hr= at 0 time under normal condition.
4hr= after 4 hr under bile condition

Bile salt hydrolase activity was determined according to Du Toit et al. (2003). After bacterial isolates refreshed for 24 h at 37 °C, they streaked on MRS agar supplemented with 0.5% w/v Oxgall bile(Merck, Germany) and incubated for 48 h at 37°C. The hydrolysis activity was determined by partial hydrolysis in comparison to the control MRS dishes.

DNA extraction and identification

The selected LAB isolate was inoculated in MRS and incubated at 37 °C overnight. Then, 800 µl broth was placed in a clean tube and centrifuged at 5160 g for 10 min. DNA of strain was extracted using a QIAamp DNA Mini Kit (Qiagen, Germany, GmbH) as described from manufacturer's instructions and the DNA was stored at -20°C. Thereafter, LAB isolate was identified by amplification and sequencing of the 16S rRNA gene. Amplification of the 16S rRNA gene was performed with the universal primer pair p8FPL (5 ′- AGTTTGATCCTGGCTCAG - 3′) and p806R (5′- TACGGYTACCTTGTTACGACTT-3′) in Applied biosystem 2720 thermal cycler (Foster City, CA, USA) under the cycling conditions: (1) initial denaturing step at 94 °C/5 min; (2) 35 cycles of denaturation (94 °C/30 s), annealing (56 °C/ 60 s) and extension (72 °C/60 s); and (3) final extension at 72 °C/12 min (Lagacé et al. 2004).

Direct sequencing was performed with a Big Dye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA). The same primers applied for PCR were also used to sequence both strands of the PCR products. Applied Biosystems 3130 genetic analyzer (HITACHI, Japan) was applied for sequencing reactions with the POP-7 system and reviewed carefully using Chromas software (Griffith University, Queensland, Australia). Nucleotide sequences were aligned using CLUSTAL X software (Larkin et al. 2007). The 16S rRNA sequences was deposited in the GenBank database of the NCBI as reference data

Determination of antibiotic resistance and virulence genes

Virulence factors and antibiotic susceptibility against antibiotics commonly used were determined in LAB isolates by detecting the presence of virulence and antibiotic resistance genes. Screening of the virulence and antibiotic determinants *efaAfm*, *esp* and *gelE*, *Asa1, cylA ermB, blaZ, van A* and *TetK* were performed by PCR amplification using the primers detailed in Table 1 following previously described procedures (Lagacé et al., 2004).

Production of white soft cheese

White soft fresh cheese samples were manufactured at the Faculty of Agricultural, Dairy Department of New Valley University according to El-Kholy et al. (2016). The control cheese samples manufactured without probiotic strain while the other cheese inoculated with the probiotic *Lb. rhamnosus* 1.5% (v/v) with approximately an initial inoculum of 8 log CFU/g^{-1}. The cheese was drained for 24hr overnight, followed by storage at 5±1 °C for 21 days.

Cheese Microbiological analysis

The presence of *E. coli* and *Staphylococcus aureus* was determined as described by the standards (ISO 11866-2, 2005 and ISO 6888-1, 2021). *Lb. rhamnosus* counts were also followed during shelf life by plating in MRS. All tests were done in triplicate.

Sensory evaluation

Ten expert panel members of the dairy science department performed sensory evaluation of cheese. The organoleptic scores consisted of appearance (10 points) body/texture (40 points), and flavor (50 points), at fresh, 15, and 21 days of cold storage.

TABLE 1. polymerase chain reaction (PCR) primer used for detection of antibiotic resistance and virulence genes

Gene	Responsible for	Sequence	Product size (bp)	Reference
efaAfm	Antigen of bacteria endocarditis	AACAGATCCGCATGAATA CATTTCATCATCTGATAGTA	735 bp	Eaton and Gasson (2001)
esp	Immune evasion	AGATTTCATCTTTGATTCTTGG AATTGATTCTTTAGCATCTGG	510 bp	Vankerckhoven et al. 2004
gelE	Hydrolysis of gelatin, collagen, haemoglobin	TATGACAATGCTTTTTGGGAT AGATGCACCCGAAATAATATA	213 bp	Vankerckhoven et al. 2004
asa1	Aggregation substance	GCACGCTATTACGAACTATGA TAAGAAAGAACATCACCACGA	375 bp	Vankerckhoven et al. 2004
cylA	Cytolysin gene	ACTCGGGGATTGATAGGC GCTGCTAAAGCTGCGCTT	688 bp	Vankerckhoven et al. 2004
ermB	Resistant to erythromycin antibiotic	CATTTAACGACGAAACTGGC GGAACATCTGTGGTATGGCG	425 bp	Moghimi et al. 2023
blaZ	Resistant to penicillin antibiotic	TACAACTGTAATATCGGAGGG CATTACACTCTTGGCGGTTTC	833 bp	Moghimi et al. 2023
vanA	Resistant to vancomycin and teicoplanin antibiotics	CATGACGTATCGGTAAAATC ACCGGGCAGRGTATTGAC	885 bp	Moghimi et al. 2023
tetK	Resistant to tetracycline antibiotic	GTAGCGACAATAGGTAATAGT GTAGTGACAATAAACCTCCTA	360 bp	Moghimi et al. 2023

TABLE 2. Showed the source of isolates, their ability to grow under different stress conditions, antibiotic resistance genes and Molecular identification.

LAB isolates	Source	Hemolysis activity	Fermentation in skim milk	Survival rate % at pH 2 after 3 h	Pepsin after 3 h	tolerate 2% bile after 4 h	Bile salt hydrolase 0.5%	Molecular identification of selected isolates
R00	*Raw milk*	ɣ	Accepted	92.93%	61.32%	NC	NC	NC
R01	*Raw milk*	ɣ	Not accepted	NC	NC	NC	NC	NC
Y03	Yoghurt	ɣ	Accepted	82.5%	88.8	92.29	+	*Enterococcus faecium*
Y04	Yoghurt	ɣ	Not accepted	NC	NC	NC	NC	NC
CH05	Karish cheese	ɣ	Not accepted	NC	NC	NC	NC	NC
CH06	Karish cheese	ɣ	Accepted	79.7%	52.76%	NC	NC	NC
CH07	Karish cheese	ɣ	Accepted	35.4	NC	NC	NC	NC
CH08	Karish cheese	ɣ	Accepted	84.86%	80%	97.4%	+	*Lacticaseibacillus rhamnosus*
CH10	Karish cheese	ɣ	Accepted	ND	NC	NC	NC	NC
CH11	Domiati cheese	ɣ	Accepted	76.69 %	44.7%	NC	NC	NC
CH12	Domiati cheese	ɣ	Accepted	56.2	NC	NC	NC	NC
12 isolates								2 isolates

Abbreviations: NC, not calculated; ND, not detected.

Results and Discussion

Isolation of LAB strains, safety assessment and fermentation

In this study, out of 80 isolates, only 20 exhibited LAB properties that are gram-positive, catalase-negative, and lactose-fermenters. The LAB isolates were also exhibited to the blood hemolysis test to assess their safety, where none of the examined isolates showed β-hemolytic. Only 12 isolates are γ-hemolysis, whereas 8 isolates showed partial hemolysis. The lack of hemolytic activity is a safety requirement to reveal potential probiotic strains (Casarotti et al., 2017). Furthermore, the absence of hemolytic activity is an indicator that the strains are free from opportunistic virulence as a safety criterion (Peres et al., 2014). Previous studies also assured the absence of hemolytic activity among LAB species (Casarotti et al., 2017). All isolates that showed no hemolysis were evaluated for their sensory characteristics according to the ability of these strains to cause acceptable fermentation in skim milk. Nine isolates showed distinguishing sensory properties for flavor and texture, while three isolates exhibited rancidity and bitterness (Table 2).

Screening for probiotic properties

In order to determine the probiotic properties, in *vitro* analyses were performed to determine the potential probiotic characteristics of the remaining nine LAB strains. Out of 9 LAB isolates, 7 were able to survive at pH 2.0. Probiotics must have the capability to survive passage through the stomach and small intestine. Tolerance of low pH in the stomach's gastric juice and the small intestine is a critical property for promising probiotic properties (Terpou et al., 2019). However, many studies also reported that LAB are widely distributed in nature and include a wide group of different genera. In addition, they are among the most important microorganisms used in food fermentations and can produce lactic acid at different rates and grow under varying conditions (Ningtyas et al., 2019).

The isolates that have the ability to survive at low pH were examined to tolerate 2% bile salts. Only two isolates showed the capability to tolerate 2% bile salts (Table 2). Strain CH08 showed the highest survivability of 97%, followed by isolate and Y03 at about 92%. Furthermore, the ability of the isolated bacteria to hydrolyze bile salts was also tested for two isolates that showed the ability to tolerate bile salts. Both Y03 and CH08 strain exhibited positive results for hydrolase bile salts. However, only the isolates that are resistant to both low pH and bile salts can enhance their survival capacity in the small intestine. Bile salts hydrolase BSH is an enzyme produced by intestinal microbiota that can deconjugate of bile acids, which are less soluble and less efficiently reabsorbed from the intestinal lumen than their conjugated counterpart (Mojgani et al., 2015). Additionally, the level of bile salts deconjugation can significantly affected on cholesterol assimilation by lactobacilli (Jones et al., 2013). In this context, previous studies have also indicated this correlation between the ability of isolates to hydrolyze bile salts and a reduction in cholesterol levels (Tomaro-Duchesneau et al., 2014).

Screening for virulence factors and antibiotic resistance genes and identification

To gain safe probiotic products for human consumption, the selected isolate has to be free from any transferable antibiotic and virulence genes. Enterococci are a typical example of transiminated genes that can be disseminated and cause the virulence of pathogenic bacteria. However, most lactobacilli are generally recognized as safe and show intrinsic resistance to aminoglycosides (gentamicin, kanamycin, neomycin, and streptomycin), vancomycin, ciprofloxacin, and trimethoprim. Nevertheless, resistance transfer from LAB, including lactobacilli, has been proven both in vitro and in vivo (Anisimova et al., 2022; Moghimi et al,. 2023). Various non-specific ways may spread these resistance determinants, such as defective cell wall autolytic systems and multidrug transporters that may be acquire resistance throughout mutation or via the acquisition of resistance genes from other organisms (wang et al., 2019).However, only two isolates, Y03 and CH08, showed positive results to enhance their survival capacity as probiotic strains. In an attempt to trace the presence of the resistance and virulence genes in our selected isolates, we amplified these determinants: *efaAfm*, *esp* and *gelE*, *Asa1, cylA,ermA, blaZ, vanA and tetK*. Interestingly, only one of the two selected LAB isolates, CH08, identified as *Lacticaseibacillus rhamnosus*, has been free from all virulence and antibiotic-determinant genes. While the other Y03 was identified as *Enterococcus faecium* has been implicated in all virulence and resistance determinants. *E. faecium* are the most common types of enterococci that are a reason for multidrug-resistant infections in hospitals (Schwartzman et al., 2023). In contrast to the

current study, Wang et al. (2019) found a high prevalence of antibiotic-resistant LAB isolated from dairy products. In addition, many different studies usually exhibit the occurrence of antibiotic resistance and virulence genes among LAB from food sources. Some studies have pointed out that the presence of vancomycin resistance gene are naturally found in many *Lactobacillus* species which is the last resort of defense against resistant bacteria (wang et al., 2019). However, other study conducted by Anisimova et al. (2022), most of the *Lb. rhamnosus* strains were found to be phenotypically and genotypically susceptible to all antibiotics tested. The results of the determination of antibiotic resistance and virulence genes are shown in Table 3.

Lb. rhamnosus CH08 wasthe only selected probiotic strain which exhibited favorable sensory properties for potential use in functional fermented foods, no blood hemolysis and is free from all tested virulence and antibiotic resistance genes. All LAB isolates were catalase and oxidase negative, heterofermentative that can produce acid and gas from glucose, and nonmotile. *Lactobacillus* is considered one of the most common types of lactic acid bacteria used as the starter culture of dairy products and can also be defined as a probiotic. Moreover, the characteristics of probiotics that are related to Lactobacilli, regardless, belong to intestinal origin (Solieri et al., 2014).

Monitoring Lacticaseibacillus rhamnosus survival

In this study, selected probiotic strains isolated from cheese were checked for viability through the storage period and their impact on the final product. *Lb. rhamnosus* was employed asanadjunct culture in white fresh cheese production, and microbial counts of probiotics during storage at a cold temperature are presented in Fig. 1. A good level was obtained with bacterial counts of selected probiotic *Lb. rhamnosus* in fresh cheese that remained above 7 log CFU/g^{-1} and ranged between 8and 9 log CFU/g^{-1} during the shelf life of the product for 21 days at cold storage. These results are also consistent with previous studies, which have recommended a range of probiotic viable counts from 6 to 7 log CFU/g^{-1} at the time of consumption to gain health benefits to humans or hosts (Terpou et al., 2019). Hence, this minimum limit of probiotics in functional products will allow an appropriate level to survive the upper ingestion and exert their beneficial effects in the human body. In this study, *Lb. rhamnosus* also exhibited the highest counts, either when fresh or at the end of the storage period. Maybe this is because the viable cell count was 8 log CFU/g^{-1} at the initial time point and using *Lb. rhamnosus* individually. These results correspond with those obtained by Triana et al. (2016). Moreover, some probiotic strains have the ability to improve cheese quality and sensory properties (Galli et al., 2019). However, it is preferable to test each probiotic strain individually to check their viability after being incorporated into curds and to determine their final effect on the product. Fresh cheeses with high pH are usually characterized by their short shelf life and the sensitivity of colonization with coliforms, *Pseudomonas* spp., yeasts and molds, and occasionally pathogens through post-processing contamination. The addition of probiotics can play an important role as beneficial supplements, improving sensory properties and biopreservative agents for extending the shelf life and controlling the spoilage of fresh cheese. Regarding the microbiological quality of both fresh probiotic cheese and control, no viable counts of spoilage bacteria, *S. aureus* and *E. coli*, were detected during shelf life. Previous studies have found that *Lb. rhamnosus* displayed high antimicrobial activity for *S. aureus* and *E. coli* (Afshari et al., 2022). Furthermore, the use of high-quality raw materials and appropriate heat treatment ensures the elimination of these types of bacteria, as shown in Fig. 1.

Coliform bacteria are generally considered an important indicator of insufficient processing stages and a lack of good manufacturing practices (GMP). Thus, their presence can reflect the hygienic status of milk production, processing, storage, and distribution systems. However, *Lb. rhamnosus* is well documented as a probiotic with a protective role, either in the prevention or control of the colonization of potential pathogens. A previous study by Johnson-Henry et al. (2018) found that *Lb. rhamnosus* has the capability to protect the epithelial cell barrier in response to challenge with the enteric pathogen *E. coli* O157:H7, while de Alcântara et al. (2019) reported that *Lb. rhamnosus* can also reduce the viability of *S. aureus* and *P. fluorescens*.

Sensory evaluation of probiotic white soft cheese

It is also interesting to mention that too much difference could be observed between the flavors of the cheeses, especially at the end of the storage period (21 days). The culture-treated cheese was

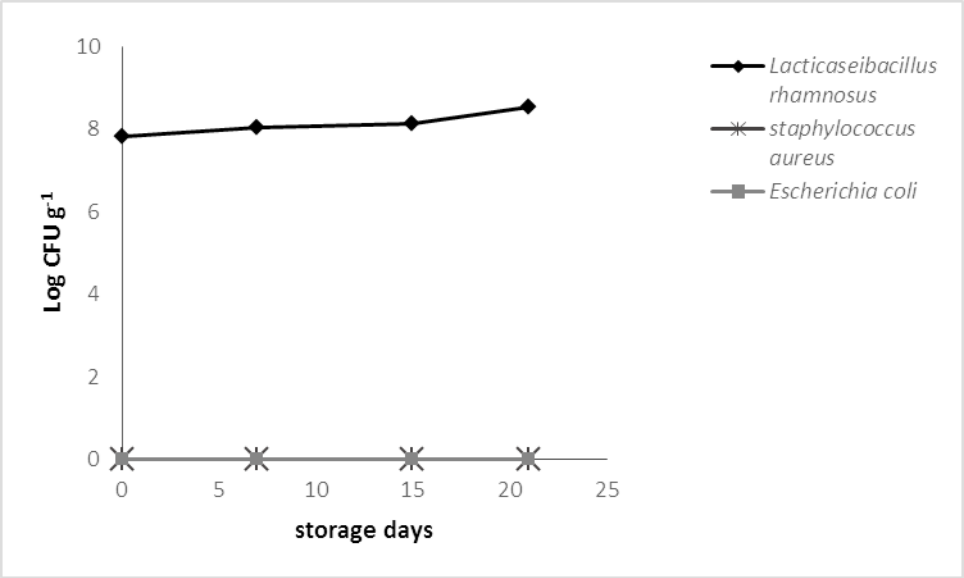

Fig. 1. Development of Viable probiotic counts (log7 CFU g^{-1}) of *Lb. rhamnosus* (□), *S. aureus* (▲) and *E. coli* (•) in fresh cheese during storage at 5 °C.

TABLE 3. Screening of virulence and antibiotic resistance genes in selected strains.

LAB Strains	Virulence and resistance genes								
	efaAfm	*Esp*	*gelE*	*Asa1*	*cylA*	*erm A*	*blaZ*	*Van A*	*tetK*
CH08 (*Lacticaseibacillus rhamnosus*)	-	-	-	-	-	-	-	-	-
Y03 (*Enterococcus faecium*)	+	+	+	+	+	+	+	+	+

superior and gave a pleasant mouthfeel flavor. The cheese flavor at the end of storage was clean and had a fully ripening taste (Table 4). These could be attributed to the heavy proteolysis in the cheese protein as expressed by the soluble nitrogen and free amino acids. Additionally, no bitterness or off-flour was recorded for culture-treated white soft cheese during the storage period. Galli et al. (2019) reported that some probiotic strains have the ability to improve cheese quality and sensory properties. However, it is preferable to test each probiotic strain individually to check their viability after being incorporated into curds and to determine their final effect on the product. On the contrary, Gomes et al. (2011) found that probiotic cheese had lower scores for appearance, aroma, and texture compared to traditional or non-probiotic cheese due to the different pathway of metabolism and synthesis of organic acids. However, *Lb. rhamnosus* has been proven to significantly enhance fermented flavor when compared to other cheeses without negatively affecting the rheological properties of dairy products (Jimenez, 2012).

Lb. rhamnosus specie is a mesophilic culture that can also grow at wide range of temperatures between 2.6 °C and 52 °C and 44.4 °C is the optimum growth temperature. Which can enhance its shelf life during cheese manufacturing and cold storage of the final product (Galli et al. 2019). Based on these results, we conclude that *Lb. rhamnosus* has shown good potential probiotic properties and ideal sensory characteristics for cheese processing. Moreover, it also confirms and points out that indigenous isolates are still an exceptional source of diverse probiotic properties regarding health benefits and cheese processing.

TABLE 4. Sensory evaluation of probiotic white soft cheese produced using Lb. rhamnosus compared with control during storage for 21 days at 5±1 °C

Cheese	Storage period (days)	Sensory properties			
		Appearance 10	Body and texture 40	Flavor 50	Overall acceptability
white soft cheese (control)	Fresh	9.0	36.0	40.0	85.0
	15	9.0	36.5	42.0	87.0
	21	8.5	37.0	43.0	88.5
Probiotic white soft cheese	Fresh	9.0	38.0	40.0	87.0
	15	9.0	38.5	45.5	93.5
	21	9.0	38.5	48.5	96.5

Conclusions

The results of the present study indicate that using indigenous strain *Lb. rhamnosus* isolated from New Valley region is considered a good strategy to improve sensory properties and add potential health benefits as a probiotic supplement in non-starter white soft cheese. Strain *Lb. rhamnosus* did not show the presence of any tested resistance genes or hemolytic activity. Furthermore, it also confirms their ability to be viable throughout the tested shelf life with high counts, which enhanced their efficacy as a technological probiotic supplement. It also showed greater acceptance when compared to the original control cheese without any significant change in the ideal product properties. Hence, the use of the strain *Lb. rhamnosus* proved their ability not only to manufacture probiotic white soft cheese but also to approach its sensorial properties in a microbiologically safe way. Therefore, this culture proved that it can be a viable strategy to improve its acceptance significantly.

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