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Research Article
Anti-*Salmonella* Potential of a Traditional Dairy Isolate of *Limosilactobacillus fermentum* in Domiati-Like Cheese

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Abstract

Background and Objective: Most chemical preservatives are considered hazardous to human health, and food manufacturers worldwide are reducing their use. Consequently, scientists in the food industry have turned to biological control applications, where protective microorganisms, which produce substances that inhibit pathogens, are among the most important biological applications. This research aimed to assess the antibacterial activities of two strains of *Limosilactobacillus fermentum*, which were isolated from traditional Egyptian dairy products (Mish and Karish cheeses), against *the Salmonella* Typhimurium pathogen in Damietta-like cheese. **Materials and Methods:** Six treatments of Damietta-like cheese were made using the inoculation of a single strain of *Limosilactobacillus fermentum* and *Salmonella* Typhimurium, as well as a control for each single strain of *Limosilactobacillus fermentum* or *Salmonella* Typhimurium. The data was a statistical analysis using One-way ANOVA, and the results were compared at a level of significance $p<0.05$. **Results:** The results confirmed a completely inhibited *S. typhimurium* in cheese using *Limosilactobacillus fermentum* DMRC 309 concentration of log 3 CFU/g in milk; however, DMRC 316 reduced the *S. typhimurium* count from log 3 to log 1.92. **Conclusion:** The *Lactobacillus fermentum* DMRC 309 has a promising application for controlling *Salmonella* and increasing dairy-food safety characteristics.

Key words: Antibacterial efficacy, Damietta-like cheese, food safety, *Limosilactobacillus fermentum, Salmonella* pathogen

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Cheese is the most globally widespread and staple dairy[1,2]. Dairy products possess significant biological and nutritional importance, making them a perfect medium for the proliferation of bacteria that may lead to food spoilage and illnesses[3]. Important public health and economic implications arise from disease outbreaks caused by consuming cheese contaminated with pathogen or their metabolites. Medicines, duties, increased production waste, company losses, product recalls, spoilage, and outbreak investigations are among the losses caused by epidemics[4,5].

It is well recognized that *Lactobacillus* species can inhibit harmful bacteria, such as *S. typhimurium*, in foods like cheese. When manufacturing cheese, using specific strains of *Lactobacillus* as secondary cultures can help prevent *S. typhimurium*. Because of their strong antibacterial properties and ability to survive in the cheese environment, these cultures can be selected[6]. The safety and quality of the cheese can be enhanced by adding *Lactobacillus* as an adjunct culture to the primary starter culture[7]. Damietta cheese is the most popular soft cheese in Egypt. It is made by adding high salt to milk before coagulation. The salt in the final products is 4-8%, but recently, low-salt cheese (2-3% salt) has been produced under other names[8]. Cheese manufacturers may improve product safety and lower the possibility of pathogen contamination, such as that caused by *S. typhimurium*, by utilizing *Lactobacillus's* inherent antibacterial qualities. This protects consumers and improves the final product's quality and shelf life[6].

This research aimed to apply promising *Limosilactobacillus fermentum* strains from traditional Egyptian dairy products to control the *S. typhimurium* in Damietta-like cheese, which has low salt. Applying the isolated *Lactobacillus* strains with high antimicrobial potency can protect public health, help maintain the cheese's quality, and extend its shelf life.

MATERIALS AND METHODS

Study area: The study was carried out at the Laboratory of Dairy Microorganisms and Cheese Research, Department of Dairy Science and Technology, Faculty of Agriculture, Alexandria University, Egypt, from December, 2023 to July, 2024.

Materials: Two strains of *Limosilactobacillus fermentum* (DMCR 309 (isolated from Mish cheese and DMCR 316 isolated from Karish cheese) were previously isolated in Laboratory of Dairy Microorganisms and Cheese Research (DMCR), full characteristics for antimicrobial properties against pathogens which included bacteria and fungus, as well as safety properties, included hemolysis, antibiotic resistance and identification by 16S rRNA and bacteriocin gent producing. *Salmonella enterica* subsp. *enterica serovar* Typhimurium CDC 6516-60 (ATCC 14028) was obtained from the American culture collection.

Cow milk for cheese making was obtained from a dairy farm, Faculty of Agriculture, Alexandria University, Egypt.

Damietta cheese, like manufacturing: Two strains were chosen for cheese manufacturing: *Limosilactobacillus fermentum* (DMCR 309 and DMCR 316), as those strains have salt tolerance (6 and 8%, respectively)[7]. It also has a significant inhibition impact against *S. typhimurium* using their cell-free extract as crude, neutralized, and catalase-treated. In this regard, strain application was targeted to assess their food safety potency and organoleptic expected characteristics. Three replicates of experimental Damietta-like cheeses for each treatment were processed. Each lactic acid bacteria (LAB) strain was grown in MRS medium (37°C/24 hrs). Then, it was inoculated at 10% in reconstituted sterile skimmed milk until coagulation. The milk was pasteurized (64°C/30 min) and cooled to 40°C. The milk was divided into six parts:

- **Group 1:** Was the negative control (without inspected *Limosilactobacillus* or *S. typhimurium*
- **Group 2:** Was positive control contaminated milk by *S. typhimurium* (log3 CFU/mL)
- **Group 3:** Was inoculated with selected DMCR 309, which had significant antibacterial activities against *S. typhimurium*
- **Group 4:** Was inoculated with selected DMCR 316, which had significant antibacterial properties against *S. typhimurium*
- **Group 5:** Was a positive control of contaminated milk by *S. typhimurium* (log3 CFU/mL) in the presence of DMCR 309
- **Group 6:** Was positive control contaminated milk by *S. typhimurium* (log3 CFU/mL) in the presence of DMCR 316

After the incubation (60 min), the calcium chloride (0.02%) salt (6% in milk) and a suitable amount of commercial calf rennet were added to coagulate the milk in 90 min[8]. The curd was then transferred to plastic containers lined with cheesecloth. After 2-3 hrs, flat weights (2-2.5 kg/10 kg of cheese milk) were placed to compact the curd. The cheese blocks were then arranged in cans and stored at a cool temperature (3-5°C/30 days).

Lactic acid bacterial count of cheese: The number of lactic acid bacteria (*L. fermentum*) was determined using a plating method using deep inoculation on the MRS medium. Plates were incubated at a temperature of 37°C for 72 hrs.

Physiochemical analysis of cheese: The moisture content of cheese samples was determined by drying the oven at 105°C until constant weight[9]. The cheese sample (20 g) was mixed with an equivalent amount of distilled warmed water (40°C), the mixture was kept for 30 min, and then the pH was recorded using a glass electrode of EPD pH meter. The titratable acidity of cheese was determined as lactic acid%[9]. The salt content in cheese samples was determined according to Volhard's method[8].

Counting and detection of *Salmonella*: *Salmonella* was enumerated, counted on XLD Agar, and incubated at 37°C for 24 hrs. Following incubation, the bacterial colonies were carefully counted. The horizontal method for detecting, enumerating, and serotyping *Salmonella* was carried out according to ISO 6579-1[10].

Statistical analysis: The data is represented as a mean of triplicates. For the ANOVA, the SAS software package[11] was used; results were compared at a level of significance $p<0.05$.

RESULTS AND DISCUSSION

Damietta cheese is the most popular soft cheese in Egypt. Based on the results, the selected *Limosilactobacillus* strains can be considered relatively safe to use as beneficial cultures in the food industry. Strains chosen for application in cheese-making to control *S. typhimurium* should be hemolysis negative, have limited resistance to a few antibiotics, have a low autolysis rate to survive in cheese during storage, and have excellent flavor in fermented milk. In addition to acid production and salt tolerance for strain selection for making Damietta-like cheese. Two bacteria were selected from the isolated strains gathered in this experiment for cheese-making. Strain application evaluated the strains' effectiveness regarding food safety and desired sensory qualities. Each treatment included the processing of three duplicates of experimental Domietta-like cheese.

Physicochemical properties of damietta like cheese: Cow milk was used to make Damietta-like cheese. The moisture level of fresh cheese ranges from 60.5 to 68.15%, as shown in Table 1. However, as the cheese is stored for one month, its moisture content dramatically drops due to biochemical changes. These results are consistent with the previous findings[8,12], who found that every cheese sample exhibited a progressive decrease in moisture during the ripening process. Significant variations were observed between the fresh cheese treatments; these variations are only attributable to the pressing of cured *S. typhimurium*-contaminated cheese but are not related to other conditions. The development of titratable acidity (TA) over the storage period was the reason behind the decrease in moisture content. Table 1 displays the variations in the titratable acidity of cheese produced using various treatments[3]. It has been observed that TA progressively increases throughout storage intervals. After a month, the little variations between the cheese treatments were noteworthy. The moisture content of the cheese treatment showed significant variations in acidity, simulating the growth rate of nonstarter bacteria and their capacity to ferment lactose throughout storage. These findings agree with those demonstrating that all cheeses become more acidic as they ripen[13].

pH and salt content of cheese during storage: The pH values of all the cheese treatments vary significantly due more to moisture content than to the addition of a protective culture or strain of *Salmonella* Typhimurium. A negative correlation exists between acidity and pH, and pH decreases during storage. Fresh cheese has a salt level between 3.11 and 3.6% depending on the moisture content, which is high in cheese with a high moisture content. After one month of storage, the salt slightly rises depending on moisture levels[7].

Inhibitory effects applied strains against *S. typhimurium* in damietta-cheese-like: The purified *Limosilactobacillus fermentum* DMCR 309 and DMCR 316 have inhibitory actions against *S. typhimurium*. *Limosilactobacillus fermentum* DMCR 309 inhibited the pathogenic bacteria at a count log 3 of *S. typhimurium* on the first day and for the storage period. No appreciable differences were observed between samples prepared without adding *Salmonella* Typhimurium

Table 1: Physicochemical analysis of Damietta cheese like during storage

		Mean of physicochemical analysis				
Treatment	Storage time (days)	pH	Acidity (%)	Moisture (%)	Salt (%)	Salt/Moisture (%)
G1	1	6.33^{a}	0.20^{h}	65.28^{b}	3.11^{f}	4.76
(negative control)	7	6.23^{b}	0.20^{h}	60.83^{c}	3.25^{e}	5.34
Just with starters	14	5.89^{f}	0.24^{e}	59.82^{cd}	3.47^{d}	5.80
	21	5.75^{h}	0.29^{d}	59.23^{d}	3.54^{c}	5.98
	30	5.70^{im}	0.30^{d}	59.33^{d}	3.55^{c}	5.98
G2	1	6.12^{c}	0.21^{h}	60.05^{cd}	3.04^{f}	5.06
(positive control)	7	6.09^{c}	0.26^{d}	60.31^{cd}	3.16^{f}	5.24
contaminated with *Salmonella*	14	6.04^{c}	0.26^{e}	60.51^{cd}	3.33^{et}	5.50
	21	6.03^{d}	0.26^{e}	60.16^{cd}	3.76^{b}	6.25
	30	6.00^{e}	0.38^{c}	59.62^{cd}	3.46^{d}	5.80
G3	1	6.04^{d}	0.24^{e}	64.13^{b}	3.38^{de}	5.27
Cheese fortified with DMCR 309	7	5.89^{f}	0.27^{de}	61.68^{c}	3.59^{c}	5.82
	14	5.99^{e}	0.28^{de}	60.09^{cd}	3.70^{b}	6.16
	21	6.02^{d}	0.29^{d}	60.54^{c}	3.86^{a}	6.38
	30	6.03^{d}	0.29^{d}	60.70^{c}	3.99^{a}	6.57
G4	1	6.00^{e}	0.27^{ef}	67.29^{a}	3.30^{e}	4.90
Cheese fortified with DMCR 316	7	6.03^{d}	0.27^{ef}	61.01^{c}	3.41^{d}	5.59
	14	5.93^{f}	0.27^{ef}	60.18^{cd}	3.52^{c}	5.85
	21	5.92^{f}	0.32^{d}	58.78^{d}	3.70^{b}	6.29
	30	6.01	0.33^{d}	57.54	2.92	5.07
G5	1	6.00^{e}	0.23^{f}	68.15^{a}	3.60^{c}	5.28
Cheese fortified with DMCR 309/	7	6.02^{d}	0.28^{de}	56.64^{e}	3.30^{e}	5.83
contaminated with *Salmonella*	14	5.94^{f}	0.28^{de}	56.60^{e}	3.54^{c}	6.25
	21	5.87^{g}	0.28^{de}	56.04^{e}	3.68^{b}	6.57
	30	5.83^{f}	0.30^{d}	56.38^{e}	3.75^{b}	6.65
G6	1	6.00^{e}	0.23^{f}	68.15^{a}	3.60^{c}	5.28
Cheese fortified with DMCR 309/	7	5.96^{f}	0.23^{f}	62.90^{c}	3.63^{bc}	5.77
contaminated with *Salmonella*	14	5.76^{h}	0.30^{d}	61.94^{c}	3.75^{b}	6.05
	21	5.73^{i}	0.42^{b}	61.90^{c}	3.87^{a}	6.25
	30	5.69^{m}	0.48^{a}	60.02^{cd}	3.95^{a}	6.58

G1 was the negative control (without inspected LAB or *S. typhimurium*, G2 was positive control contaminated milk by *S. typhimurium* (log3 CFU/mL), G3 was inoculated with selected DMCR 309, which had significant antibacterial activities against *S. typhimurium*, G4 was inoculated with selected DMCR 316, which had significant antibacterial properties against *S. typhimurium*, G5 was positive control contaminated milk by *S. typhimurium* (log3 CFU/mL) in presence with DMCR 309, G6 was positive control contaminated milk by *S. typhimurium* (log3 CFU/mL) in presence with DMCR 316, The acidity value was determined as a lactic acid percentage and Different letters in the same column denote significant differences ($p<0.05$)

and samples prepared with the addition of *Salmonella* Typhimurium and a protective culture of *Limosilactobacillus fermentum* DMRC 309. Although the sample containing both *S. typhimurium* and *Limosilactobacillus fermentum* DMRC 316 did not entirely suppress the bacteria, there was a 50% reduction in the number of *S. typhimurium* on the first day, and this inhibition increased after the Damietta were stored. Cheese manufacturers can make their products safer by utilizing *Limosilactobacillus'* inherent antibacterial qualities, which lower the possibility of infection by pathogens like *S. typhimurium*. This helps preserve the cheese's quality and shelf life and safeguards customers. *S. typhimurium* and other harmful bacteria are known to be controlled by *Limosilactobacillus* species in food products like cheese[14]. The two main antibacterial strategies against *S. typhimurium* are pH reduction and direct inhibition. *S. typhimurium* can be directly inhibited from growing by the acids, bacteriocins, and hydrogen peroxide that *L. fermentum* produces. These antimicrobial substances disrupt the pathogen's cellular integrity and metabolic functions, inhibiting or killing the organism. Additionally, *S. typhimurium* inhibition is linked to pH reduction because the bacterium likes a neutral pH for optimal growth, and the synthesis of lactic and other organic acids decreases the pH of the surrounding environment[15].

Salmonella strains, when acting as pathogens, can create very toxic compounds that may compromise the safety of food products. The properties of this product align with other risks found in dairy products, such as toxigenic fungus. Various bacterial species have shown the presence of enzymes capable of breaking down food toxins into substances that are either less harmful or completely harmless[16]. Enzymatic

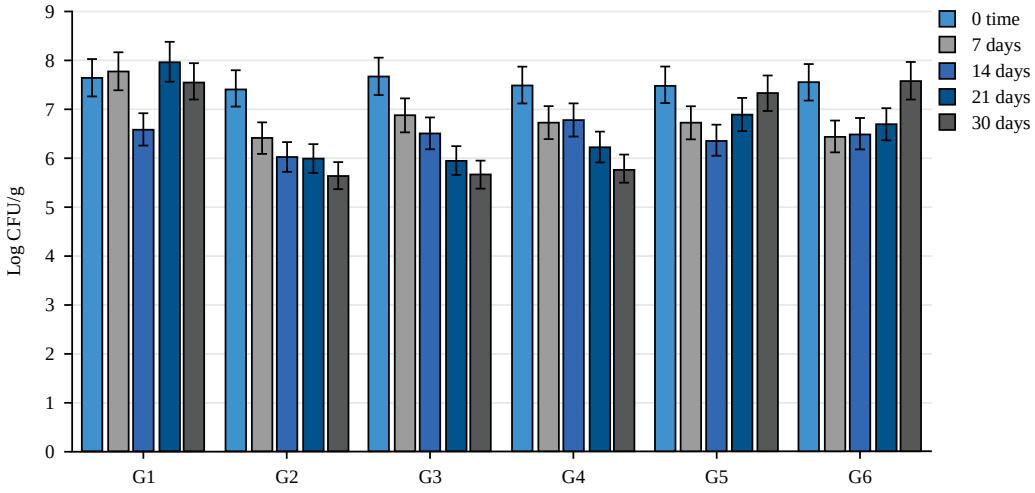

Fig. 1: Log CFU/g of the LAB count in cheese samples during storage for the control, bacterial-fortified and *Salmonella*-contaminated samples

activity may reduce the toxicity of food and feed products that have been polluted. Bacterial metabolites, especially those produced by probiotic bacteria, can help decontaminate by several methods. The findings demonstrate fluctuations when administering whole bacteria or metabolites to the specific products. The introduction of bacterial cells into food products had a vital role in affecting the growth of fungi and limiting the establishment of their metabolism[17]. Specific helpful bacteria can outperform detrimental microbes, such as fungi, that generate poisons to get nutrients and take up physical space. The occurrence above is often known as competitive exclusion. The second mechanism may be associated with the phenomena of antagonism. Some specific bacterial species can produce chemicals with antagonistic properties, which hinder the growth of infections[18]. The metabolites can have many effects, such as the disturbance of cell membranes, interference with metabolic processes, and the synthesis of enzymes that degrade toxin formation[19].

Log of LAB colony forming units in Damietta like cheese: The results shown in Fig. 1 represented the change in lactic acid bacterial count during the storage period of the produced cheese sample. It was noticed that, by the time of the storage for the manufactured cheese samples, the total count of the LAB was reduced in samples manufactured using *L. fermentum* DMCR strains (G3 and G4). These results could indicate autolysis occurring for the bacteria and bioactive released in the product.

Otherwise, the control positive sample of cheese, which was contaminated by *S. typhimurium* without DMCR-fortified strains, showed a dramatic reduction in the logarithmic count of the LAB content. This result may refer to the antagonistic effect between the LAB starter utilized for cheese manufacturing and its contaminated *S. typhimurium* content. By the end of the storage period of the contaminated cheese sample (30 days), their content of LAB was at its lowest value (Fig. 1).

Once more, cheese samples of G5 and G6 contained the *L. fermentum* DMCR strains alongside its *S. typhimurium* contamination, shown by a reduction of LAB content until 14 days of storage, followed by an increment of LAB content up to the end of the storage time (30 days). This result could be illustrated regarding the ability of fortified DMCR strains in the cheese samples to resist *S. typhimurium* contamination and antagonize its impact over time to elevate the safety properties. This suggestion may be supported by the results in Table 2, where the assay for detecting *S. typhimurium* presence in the fresh samples showed a limited presence, log 1.92 CFU/g using the strain DMCR 316 (G6), and disappeared for cheese samples using DMCR 309 (G5). Many studies of LAB isolation from a variety of foods were used as probiotic cultures and food preservatives. To prolong shelf life and guarantee food safety, bio preservation makes use of advantageous microorganisms and/or their byproducts. Several studies have shown that LAB strains naturally reduce the growth of bacteria and aid in the preservation of food products[20].

Table 2: Count and detection of *Salmonella* Typhimurium in Damietta-like cheese samples during storage

Treatment	Storage (days)	Mean *Salmonella* count (log CFU/g)	*Salmonella* detection (25 g cheese)
G1	1	ND	ND
(negative control)	7	ND	ND
Just with starters	14	ND	ND
	21	ND	ND
	30	ND	ND
G2	1	3.38[a]	+ + + + + +
(positive control)	7	3.38[b]	+ + + + + +
contaminated with *Salmonella*	14	3.09[c]	+ + + + +
	21	3.05[d]	+ + + + +
	30	2.14[f]	+ + + +
G3	1	ND	ND
Cheese fortified with DMCR 309	7	ND	ND
	14	ND	ND
	21	ND	ND
	30	ND	ND
G4	1	ND	ND
Cheese fortified with DMCR 316	7	ND	ND
	14	ND	ND
	21	ND	ND
	30	ND	ND
G5	1	ND	ND
Cheese fortified with DMCR 309/	7	ND	ND
contaminated with *Salmonella*	14	ND	ND
	21	ND	ND
	30	ND	ND
G6	1	1.92[f]	+ +
Cheese fortified with DMCR 316/	7	1.92[f]	+ +
contaminated with *Salmonella*	14	1.80[g]	++
	21	1.47[h]	+
	30	1[i]	+

G1 was the negative control (without inspected LAB or *S. typhimurium*, G2 was positive control contaminated milk by *S. typhimurium* (log3 CFU/mL); G3 was inoculated with selected DMCR 309, which had significant antibacterial activities against *S. typhimurium*, G4 was inoculated with selected DMCR 316, which had significant antibacterial properties against *S. typhimurium*, G5 was positive control contaminated milk by *S. typhimurium* (log3 CFU/mL) in presence with DMCR 309. G6 was positive control contaminated milk by *S. typhimurium* (log3 CFU/mL) in presence with DMCR 316, ND: Not detected and *Salmonella* detection represented as (-) for absence and (+) for found

CONCLUSION

Two high-salt tolerance *Limosilactobacillus fermentum* strains (DMCR 309 and DMCR 316) are employed to treat Damietta-like cheese with *Salmonella* Typhimurium. Both *Limosilactobacillus* strains do not affect the physicochemical properties of cheese but highly inhibit the *Salmonella* Typhimurium ATTC 14028, and strain *Limosilactobacillus fermentum* DMCR 309 showed significant anti-*Salmonella* contamination effects. Future studies will be designed to understand the long-term stability and efficacy of these bioprotective strains under different storage conditions, such as different brine concentrations and packaging materials, and also investigate the potential effect of combining bioprotective cultures with other natural preservatives that may improve the safety and quality of Damietta cheese.

SIGNIFICANCE STATEMENT

This study discovered the promising antimicrobial potential of lactic acid bacteria strain DMCR 309 that can be beneficial for enhancing the microbial safety and sensory quality of soft cheese. The strain was isolated from traditional Egyptian dairy products and demonstrated excellent survival under cheese-making conditions, along with desirable technological traits such as salt tolerance, acidification capacity, and flavor compound production. Notably, strain DMCR 309 completely inhibited *Salmonella* contamination in fresh cheese, highlighting its application as a natural protective culture. In contrast, strain DMCR 316 showed only partial inhibition. This study will help the researchers to uncover the critical areas of biological preservation in dairy products that many researchers were not able to explore. Thus, a new theory on natural food safety enhancers may be arrived at.

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