Influence of Probiotic Bacteria on Some Pathogenic Bacteria in Yoghurt and Kariesh Cheese

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Abstract:

Three experiments were performed to know the efficacy of probiotic (*L. rhamnosus)* on foodbornepathogens (*E. coli* O26:H11*and S. aureus*) in yoghurt and kariesh cheese. The aim of the first experiment was to investigate the ability of probiotic strain to inhibit these foodborne pathogens using agar well diffusion method, the results are the mean of three independent replicates and revealed that *L. rhamnosus* inhibited all of the tested organisms. *L. rhamnosus* had the strongest effect on *S. aureus* followed by *E. coli* O26:H11.Where the widest inhibition zone was observed in case of *S. aureus* and at the same time, the narrowest inhibition zone was observed in case of *E. coli* O26:H11.

The aim of the second experiment was to test the inhibition of *E. coli* O26:H11 and *S. aureus* in the presence of *L. rhamnosus* during fermentation under controlled pH conditions, during yoghurt manufacture. Although starter culture was the same which added to all yoghurt batches and pH levels of each kind of yoghurt were nearly the same, the difference in *E. coli* O26:H11 and *S. aureus* counts between regular yoghurt batches inoculated with *E. coli* O26:H11or *S. aureus* only and batches inoculated with *S. aureus* or *E. coli* O26:H11 plus *L. rhamnosus* may due to additional antibacterial substances produced by *L. rhamnosus*. We noticed that the elimination times of *E. coli* O26:H11 or *S. aureus* in yoghurt batches inoculated with *L. rhamnosus* more shorter than yoghurt batches inoculated with*E. coli* O26:H11 or *S. aureus* alone. The results indicated that the probiotic culture is capable of delaying growth of many foodborne pathogens, and confirmed the antagonistic effect of co-inoculation of *L. rhamnosus* with yoghurt starter against *E. coli* O26:H11and *S. aureus* during yoghurt manufacture.

The aim of the third experiment was to test the inhibition of *E. coli* O26:H11and *S. aureus* in the presence of *L. rhamnosus*, during kariesh cheese manufacture. The difference in *E. coli* O26:H11 and *S. aureus* counts between kariesh cheese batches inoculated with *E. coli* O26:H11or *S. aureus* only and batches inoculated with *E. coli* O26:H11or *S. aureus* plus *L. rhamnosus* may due to additional antibacterial substances produced by *L. rhamnosus*. From these results we noticed that the elimination times of *E. coli* O26:H11 or *S. aureus* in kariesh cheese batches inoculated with *L. rhamnosus* more shorter than kariesh cheese batches inoculated *E. coli* O26:H11 or *S. aureus* alone. Results indicated that the probiotic culture is capable of delaying growth of *S. aureus* and*E. coli* O26:H11, in kariesh cheese.

Keywords: *S. aureus*; *E. coli*; Yoghurt; Kariesh cheese; probiotic;*L. rhamnosus.*

1. Introduction

Good health starts with good nutrition, and good nutrition can protect against diseases later in life.So, consumers are looking for natural and processed foods prepared without chemical preservatives that will fit in their healthy lifestyle.

Yoghurt is a valuable adjunct to any healthy diet. It has a therapeutic value as it proved to prevent the intestinal putrefaction, gastrointestinal disorders, coronary heart diseases, reduce the risk of colon

cancer, exert a hypocholesterolemic effect and produce antibiotics as acidophilin, lactocidin, nicin and lactoline that inhibit the growth of many pathogens **(Elson and Haas, 2005)**.

Kariesh cheese is one of the most popular local types of fresh soft cheese in Egypt. It has high protein, low fat and low price. Also, it isrich in calcium and phosphorus which essential for bones and teeth as well as sodium and potassiumwhich play an important role in the formation of body liquids and muscles **(Francois *et al.,* 2004).**

The traditional method for yoghurtand kariesh cheese production offers many opportunities for microbial contamination. They generally made from raw skim buffalo's or cow's milk which is often of poor bacteriological quality owing to the high microbial load present in raw milk and the unsatisfactory conditions under which it is produced. Also, kariesh cheese is sold uncovered and without container where the risk of contamination is high. Therefore they can be considered as a good medium for the growth of different types of spoilage and pathogenic microorganisms**(Brooks *et al.,* 2012).**

E. coli frequently contaminate food and it is a good indicator for fecal pollution**.**Shiga toxin-producing *E. coli* (STEC) strains are a diverse group of food borne pathogens, including enterohaemorrhagic *E. coli* (EHEC), that are responsible for diseases in humans such as diarrhea, hemorrhagic colitis (HC), and hemolytic uremic syndrome (HUS). The most important natural reservoirs of (STEC) are cattle**(Anonymus, 2010).**

S. aureus food poisoning is one of the most common food borne disease resulted from the ingestion of staphylococcal enterotoxins (SETs), already preformed in food which have super antigenic activity whereas half of them have been proved to be emetic, representing a potential health hazard for consumers **(Hennekinne *et al.,* 2012).**

Lactic acid bacteria have an important role in limiting *E. coliand S. aureus* growth and toxin production in yoghurt and cheese which can act by bacteriocins, lactic acid production, and a decrease in pH during fermentation. Lactic acid is a weak organic acid produced during fermentation and can disturb pH homeostasis of bacteria resulting in stressed cells **(Cogan and Beresford, 2002).**

Probiotic lactic acid bacteria (LAB) include (*L. acidophilus, L. jhnsonii, L. casei, L. rhamnosus, L. gasseri* and *L. reuteri*). Lactobacilli can produce different antimicrobial components including organic acids (lactic, acetic, propionic acids), hydrogen peroxide, carbon dioxide, low-molecular weight antimicrobial substances, bacteriocins and adhesion inhibitors and thus have gained prominence as probiotics Stiles**.***L. rhamnosus* have the strongest human health efficacy data with respect to management of lactose malabsorption, rotaviral diarrhea **(Shah, 2006).**

In recognitionof public health and economic significance of these microorganisms, the present study was undertaken to investigatethe inhibitory effect of *L. rhamnosus* on the survivability of *S. aureus* and *E. coli* O26:H11 in yoghurt and kariesh cheese as food models.

2. Materials and Methods

First experiment: To investigate the ability of probiotic strain to inhibit*S. aureus* and*E. coli* O26:H11 as foodborne pathogens.

2.1. Test strains and growth conditions: Two foodborne pathogenic strains were used in this study: *S. aureus* and *E. coli* O26:H11 (which were locally isolated & identified and well-characterized in Food Control Lab. Zagazig University). *L. rhamnosus* strain was used to assess inhibitory capabilities against these pathogens in vitro. Prior to experiment, subcultures were

performed for *S. aureus* and *E. coli* on brain heart infusion (BHI) medium at 37 °C / 24 hrs and *L. rhamnosus* was cultured on Broth De Man, Rogosa, and Sharpe medium (MRS, pH 6.8, BD, Le) anaerobically at 37 °C /48 hrs.

2.2.Preparation of culture supernatant:*L. rhamnosus* was anaerobically grown in MRS broth for 24 hrs the turbidity of the broth culture was then adjusted to equal #1MacFarland standard. A cell free supernatant was obtained through centrifugation at 5000 rpm for 15 minute at 4º C, followed by sterilization through a 0.2 µm pore size filter then divided into two parts, one was adjusted to pH 7 using 0.1 N NaoH sterile solution (neutralized) and the other was left as it is (acidified).

2.3.Antimicrobial activity: For detection of antagonistic activity, agar well diffusion method was used following **(Kim and Rajagopal, 2001)** with slight modifications. Three to five colonies of each test strains were picking and suspended in trypticase soy broth (TSB)tubes then incubated overnight. The turbidity of the broth cultures were adjusted to equal #0.5 MacFarland. A sterile cotton swab was dipped into the broth suspension and rotated several times on the inside wall of the tube above the fluid level to remove excess inoculums from the swab. Mueller -Hinton agar plate was inoculated by streaking the swab over the surface with rotation of the plate for 60 degrees for three times. Wells of 5 mm diameter were cut into agar plates and 100 µl of culture supernatant fluid containing antibacterial activity were added to each well, discs of ampicillin were used as positive control. The plates were then incubated at optimum growth temperature of the indicator strains and examined after 24 hrs for inhibition zone.

2.4.Second experiment: Foodborne pathogens inhibition was tested in the presence of *L. rhamnosus* during yoghurt fermentation under controlled pH conditions.

2.4.1. Test strains:*S.aureus* and *E. coli* O26:H11 were inoculated into fresh (TSB) then incubated at 37°C /overnight. *L. rhamnosus* was inoculated into (MRS) broth then incubated anaerobically at 37 °C /24 hrs.

2.4.2.Starter culture: Defined yoghurt starter culture (lyophalized culture for direct vat set (DVS) type *L. delbrueckii sub sp. bulgaricus* and *Strep. sub sp. thermophilus* (YC-280) was used in yoghurt manufacture. The culture was kindly obtained from Chr. Hansen Laboratories, Copenhagen, Denmark.

2.4.3.Yoghurt production: Six batches of yoghurt were prepared using the procedure described by **The Egyptian Standard (1990)** from sterile (UHT) milk which preheated to 40-44°C and inoculated with yoghurt starter culture.

For regular yoghurt: One batch was inoculated with the starter culture only.

For *E. coli* O26:H11:One batch was inoculated with 10^5 CFU/ml *E. coli* O26:H11andanother one with 10^5 CFU/ml*E. coli* O26:H11 and 10^8 CFU/ml *L. rhamnosus*.

For *S. aureus*: One batch was inoculated with 10^5 CFU/ml *S. aureus*, another one with 10^5 CFU/ml *S. aureus*and 10^8 CFU/ml *L. rhamnosus*.All bacterial concentrations were done using spectrophotometer (at 600 nm wave length)

For the Probiotic: Last batch was inoculated with starter culture and 10^8 CFU/ml *L. rhamnosus* to serve as a control.All batches were incubated at 43 °C for 3 - 4 hrs until the final pH reached 4.6.

After manufacturing, the infected yoghurt and control yoghurt were stored at 4°C. Samples were taken for pH measurement and enumeration of *S. aureus* and *E. coli* O26:H11 at (0, 4, 8 hrs), (1, 3,7,14 d.) after inoculation.

2.5.Third experiment: Foodborne pathogens inhibition was tested in the presence of *L. rhamnosus* during kariesh cheese manufacture.

2.5.1. Test strains: Preparation of test strains as described in second experiment.

2.5.2- Kariesh cheese manufacture: Six batches of kariesh cheese were prepared using the procedure described by **(Aldo *et al.*, 2013)** with slight modifications. Two batches were done using *E. coli* O26:H11 negative raw milk which was put directly into special earthenware pots, which are kept undisturbed in a suitable place to allow the fat to rise to the surface forming a cream layer and the partially skimmed milk sours and clots. Then the cream layer is removed, and the curd is poured onto a mat which is tied and hung with its contents, to allow the whey drain until the desired texture of the cheese is obtained. Finally, the cheese is cut into suitable pieces, and salted (5- 7% NaCl).Another two batches of kariesh cheese were prepared as described before with the using of *S. aureus* negative raw milk.

For regular kariesh cheese: One batch was done without any inoculations.

For probiotic: Last batch was used as control inoculated with 10^8 CFU/ml*L. rhamnosus* only.

For *E. coli* O26:H11*:*One batch was inoculated with 10^5 CFU/ml *E. coli* O26:H11and another one with10^5 CFU/ml *E. coli* O26:H11and 10^8 CFU/ml *L. rhamnosus*.

For *S. aureus*: One batch was inoculated with 10^5 CFU/ml *S. aureus*, another one with10^5 CFU/ml *S. aureus*and 10^8 CFU/m *L. rhamnosus*.After manufacturing, all batches were stored at 4°C. Samples were taken for enumeration of *S. aureus*and *E. coli* O26:H11 at (0, 8, 24, 36 hrs), (2, 3, 5, 7 d.) after inoculation. The samples were prepared for examination according to **(APHA, 2004).**

Statistical analysis: Experiments were replicated three times. Average values of replications were calculated. All data were analyzed using the descriptive statistics (descriptive) &**(SPSS 14.0 Production Mode Facility Evaluation Version).**

3. RESULTS and DISCUSSION

In-vitro antimicrobial activity of probiotic:

Table (1) showed in-vitro microbial inhibition of selected pathogens using *L. rhamnosus* acidified and neutralized supernata. These results revealed that *L. rhamnosus* inhibited all of the tested indicator microorganisms either (*S. aureus*) or (*E. coli O26:H11*), the diameter of the inhibition zone of acidified supernata for *S. aureus* was 11.00 ± 1.60 mm at pH 4.90 ± 0.30 and was 9.00 ± 0.80 mm at pH 7.00 ± 0.20 of neutralized supernata, both of them were compared with positive control (Ampicillin) which was 13.00 ± 1.80 mm, while for *E. coli* was 10.00 ± 1.30 mm at pH 4.90 ± 0.30 for acidified supernata and was 8.00 ± 0.60 mm at pH 7.00 ± 0.20 of neutralized supernata, both of them were compared with positive control (Ampicillin) which was 12.00 ± 1.60 mm, there is significant inhibition in both cases. These similar results were obtained by **El-Shenawy *et al*. (2017).**

Table (1): In-vitro microbial inhibition of selected pathogens using *L. rhamnosus* acidified and neutralized supernata.

Test microorganisms	Inhibition Zone (MM)$ ± SE		
	Acidified supernata	Neutralized supernata	Positive control
S. aureus	11.00 ± 1.60*	9.00 ± 0.80*	13.00 ± 1.80
E. coli O26:H11	10.00 ± 1.30*	8.00 ± 0.60*	12.00 ± 1.60

* Significant results ($p<0.05$)$ Results are the mean of three independent trails

The primary antimicrobial effect exerted by LAB is the production of lactic acid and reduction of pH. In addition, LAB produce various antimicrobial compounds, which can be classified as low-molecular-mass (LMM) compounds such as hydrogen peroxide (H_2O_2), carbon dioxide (CO_2), diacetyl (2,3-butanedione), uncharacterized compounds, and high-molecular-mass (HMM) compounds like bacteriocin. All of these can antagonize the growth of some spoilage and pathogenic bacteria in foods **(Aween *et al*., 2012).**

Lactobacillus spp. as a probiotic can tolerate a wide range of pH (1-9) and grow well at acidic pH (1-5) **(Chowdhury *et al*., 2012)**.The inhibition of bacteria was not due to acid or low pH, because the supernatants were neutralized before testing against the organisms.

The inhibitory activity of the tested probiotic strain (*L. rhamnosus*) was slightly less against *E. coli* as compared to that obtained against *S. aureus*, indicating that *E. coli* could be less sensitive, the Gram-positive bacteria were inhibited more than the Gram-negative bacteria **(Fijan, 2016).**

Antimicrobial inhibition of *E. coli* O26:H11 and *S. aureus* by addition of *L. rhamnosus*:

L. rhamnosus is one of the widely used commercial probiotic strains with recognized health benefits, is frequently used in infant's formulas and children's food because of their preventive and curative effects on diarrhea, dental caries and allergies**(Canbulat and Ozcan, 2007)**

Concentration of 10^6–10^9 viable cells/g of a functional food product is the minimum necessary for its ingestion to have a beneficial effect **(Ouwehand *et al*., 2002).**

The ability of lactic acid bacteria to inhibit the growth of pathogenic bacteria is well known. Lactobacilli are able to compete with pathogenic bacteria when they were incubated together, but the degree of inhibition was bacterial strain depended **(Tallent *et al*., 2012).**

A – Plain yoghurt:

Table (2) summarized *E. coli* O26:H11 and *S. aureus* counts in Yoghurt manufactured with and without *L. rhamnosus* (10^8 CFU/ml) inoculation as following:

Yoghurt batch inoculated with *E. coli* O26:H11(10^5 CFU/ml) alone gave counts of $8.40 \times 10^5 \pm 0.20 \times 10^4$, $5.60 \times 10^3 \pm 0.90 \times 10^2$, $4.60 \times 10^3 \pm 1.20 \times 10^2$, $2.90 \times 10^2 \pm 1.10 \times 10$, $1.80 \times 10^2 \pm 0.80 \times 10$ and $3.60 \times 10 \pm 0.50 \times 10$ at 0, 4 and 8 h, 1, 3 and 7 d after inoculation, respectively, but it failed to be detected at 14 d after inoculation, respectively. While, yoghurt batch inoculated with *E. coli* O26:H11 (10^5 CFU/ml) and *L. rhamnosus* (10^8 CFU/ml)gave counts of $9.30 \times 10^5 \pm 1.40 \times 10^4$, $7.20 \times 10^2 \pm 5.10 \times 10$, $3.10 \times 10^2 \pm 0.70 \times 10$, $4.40 \times 10 \pm 0.80 \times 10$ at 0, 4 and 8 h, 1 d after inoculation, respectively, but it failed to be detected at 3, 7 and 14 d after inoculation as shown in fig. (1).

Table (2): Antimicrobial inhibition of *E. coli* O26:H11 and *S. aureus* in yoghurt by addition of *L. rhamnosus*.

	Time						
	0h	**4h**	**8h**	**1d**	**3d**	**7d**	**14d**
E. coli O26:H11 alone(10^5 CFU/ml)	8.40×105 ± 0.20×104	5.60×103± 0.90×102	4.60×103 ± 1.20×102	2.90×102± 1.10×10	1.80×10± 0.80×10	3.60×10± 0.50×10	-VE
E. coli O26:H11 (10^5 CFU/ml) + *L. rhamnosus* (10^8 CFU/ml)	9.30×105 ± 1.40×104	7.20×102± 5.10×10	3.10×102± 0.70×10	4.40×10± 0.80×10	-VE	-VE	-VE
S. aureus alone (10^5 CFU/ml)	7.90×105± 0.60×104	9.20×104 ±1.40×103	7.50×103 ± 3.10×102	6.20×10± 4.10×10	4.50×10± 0.20×10	-VE	-VE
S. aureus (10^5 CFU/ml) + *L. rhamnosus* (10^8 CFU/ml)	8.60×105±0 .90×104	8.50×103 ±1.90×10	3.80×10± 0.30×10	-VE	-VE	-VE	-VE

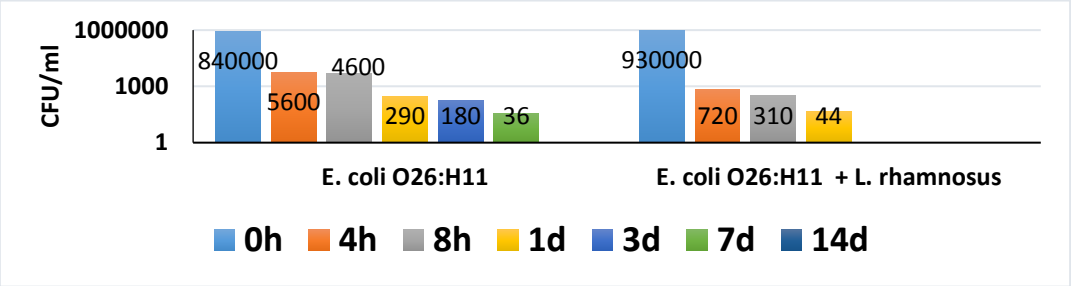

Figure (1): Count reduction and elimination of *E. coli* O26:H11 in yoghurt with/without inoculation of *L. rhamnosus*.

From the previous results we noticed that the elimination times of *E. coli* in yoghurt with *L. rhamnosus*more shorter than *E. coli* alone in regular yoghurt.

E. coli lost its viability rather slowly during refrigerated storage. Pathogenic *E. coli* organisms are significantly more acid-tolerant than non- pathogenic strains, the ability of *E. coli* to survive in high-acid food (as in the case of yoghurt) is of public health significance. (**Massa *et al.*, 1997)**

Studies of the fate of *E. coli* in (TSB) adjusted to different pH values with HCl or lactic acid revealed that the minimum pH for growth was between 4.0 and 4.5 or 4.6, respectively **(Glass *et al.*, 1992).**

Although starter culture was the same and pH levels of each kind of yoghurt were nearly the same, the difference in *E. coli* counts between regular yoghurt and yoghurt inoculated with LAB may due to additional antibacterial substances **(Kasımoğlu and Akgün, 2004)** and the usual practice was to add yoghurt bacteria (*S. thermophilus* and *L. del-brueckii ssp. bulgaricus*) to probiotic products in order to reduce the fermentation time (**Shihata and Shah, 2000**).

Also table (2) showed that yoghurt batch inoculated with *S. aureus* (10^5 CFU/ml) alone gave counts of $7.90\times10^5 \pm 0.60\times10^4$, $9.20\times10^4 \pm 1.40\times10^3$, $7.50\times10^3 \pm 3.10\times10^2$, $6.20\times10^2 \pm 4.10\times10$ and $4.50\times10 \pm 0.20\times10$ at 0, 4 and 8 h, 1 and 3 d after inoculation, respectively, but it failed to be detected at 7 and 14 d after inoculation. While, yoghurt batch inoculated with *S. aureus* (10^5 CFU/ml) and *L. rhamnosus* (10^8 CFU/ml)gave counts of $8.60\times10^5 \pm 0.90\times10^4$, $8.50\times10^3 \pm 1.90\times10$ and $3.80\times10 \pm 0.30\times10$ at 0, 4 and 8 h after inoculation, respectively, but it failed to be detected at 1, 3, 7 and 14 d after inoculation, as shown in fig. (2)**.**

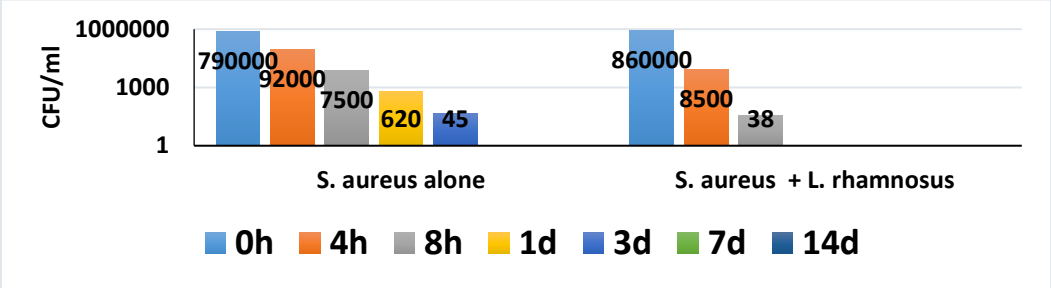

Figure (2): Count reduction and elimination of *S. aureus* in yoghurt with/without inoculation of *L. rhamnosus.*

From the previous results we noticed that the elimination times of *S. aureus*with *L. rhamnosusin* regular yoghurt more shorter than *S. aureus*alone in regular yoghurt.

S. aureus grows at a wide temperature range between 6 to 48°C with optimum of 35 to 41°C. It tolerates a pH between 4 to 10 with optimum of 6 to 7, a salt concentration of 0 to 20%, and a water activity (aw) level of 0.83 to 0.99 with optimum at 0.99. These growth characteristics enable the bacterium to grow in a wide range of foodstuffs including milk and dairy products **(Cretenet *et al.*, 2011**).

Although, the yoghurt starter cultures have inhibitory effect on *S. aureus* and can reduce the number added to milk by 1-2 log units during the cold storage **(Pazakova *et al.*, 1997).**Itcould remain viable for 3 days at low inoculums (10^3 CFU/g) and for 10 days at high inoculum (10^5 CFU/g) at refrigerated storage**.** Thus, at high contamination levels with *S. aureus* the antibacterial effect of yoghurt was insufficient to avoid the risk of food poisoning (**Mohamed and Mazyed, 2015**).

Probiotics are widely used in fermented food especially yoghurt which considered safe organisms when added to yoghurt. The lactic acid that was produced from the fermentation of lactose contributed the sour taste of yoghurt by decreasing its pH **(Heller, 2001).**

A lot of researchers reported that yoghurt could be contaminated by *S. aureus* and survived fermentation during storage period till 8-10 days. In this period, toxins could be formed and persist after inhibition of the organism by low pH. Also, by using probiotic starter culture the population of *S. aureus* could be lowered to non-detectable level within short time of storage. The hygienic quality practices were necessary to be applied in the processing, handling and transferring of the product to avoid the contamination of food with *S. aureus* and eventual production of its enterotoxins **(Salvatierra *et al*., 2004).**

Fig. (3) showed pH level change during fermentation and cold storage of regular yoghurt and yoghurt fermented with co-inoculation of *L. rhamnosus*that declared non-significant reduction in pH obtained by inoculation of *L. rhamnosus* into yoghurt with regular starter culture.

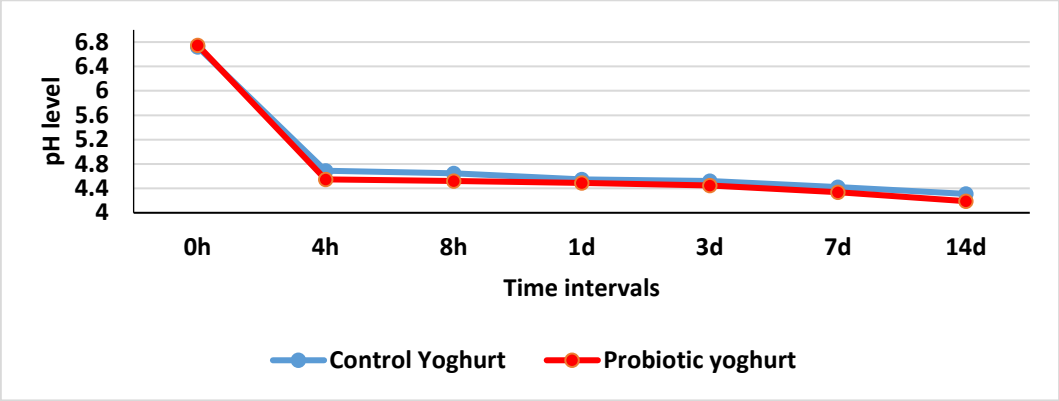

Figure (3): pH level change during storage of control and probiotic yoghurt samples.

Probiotic yoghurt exhibited slight pH drop compared with regular yoghurt direct after incubation time and during the refrigerated storage period. This decrease in pH value is due to yoghurt starter culture (*Streptococcus thermophilus* and *Lactobacillus delbruekii spp. bulgaricus*) and metabolic activity of probiotic **(Kamal *et al.*,2016).**

B- Kariesh cheese:

Table (3) summarized *E. coli* O26:H11 and *S. aureus* counts in kariesh cheese manufactured with and without *L. rhamnosus* (10^8 CFU/ml) as following:

Table (3): Antimicrobial inhibition of *E. coli* O26:H11 and *S. aureus* in Kariesh cheese by addition of *L. rhamnosus.*

	Time							
	0h	**8h**	**24h**	**36h**	**2 d**	**3 d**	**5d**	**7 d**
***E. coli* O26:H11 alone (10^5 CFU/ml)**	$3.60\times10^5\pm 0.70\times10^4$	$8.20\times10^4\pm 1.20\times10^2$	$7.10\times10^3\pm 2.30\times10^2$	$5.20\times1\pm 2.00\times10^2$	$4.70\times10^3\pm 1.70\times10^2$	$3.60\times10^2\pm 0.60\times10^2$	$5.90\times10\pm 0.90\times10$	$4.80\times1\pm 0.60\times10$
***E.coli* O26:H11 (10^5 CFU/ml)**	$6.80\times10^5\pm 0.30\times10^4$	$7.10\times10^3\pm 1.50\times10^2$	$4.10\times10^2\pm 0.80\times10$	$3.50\times10\pm 0.20\times10$	-VE	-VE	-VE	-VE
***S. aureus* alone (10^5 CFU/ml)**	$9.70\times10^5\pm 1.50\times10^4$	$2.50\times10^4\pm 2.10\times10^3$	$8.40\times10^3\pm 1.20\times10^2$	$4.20\times10\pm 0.90\times10^2$	$3.20\times10\pm 0.80\times10$	$5.60\times10\pm 0.60\times10$	-VE	-VE
***S. aureus*(10^5 CFU/ml) + *L. rhamnosus* (10^8 CFU/ml)**	$6.60\times10^5\pm 1.30\times10^4$	$7.40\times10^3\pm 3.20\times10$	$7.20\times10\pm 1.30\times10$	-VE	-VE	-VE	-VE	-VE

kariesh cheese batch inoculated with *E. coli* O26:H11(10^5 CFU/ml) alone gave counts of $3.60\times10^5 \pm 0.70\times10^4$, $8.20\times10^4 \pm 1.20\times10^2$, $7.10\times10^3 \pm 2.30\times10^2$, $5.20\times10^3 \pm 2.00\times10^2$, $4.70\times10^3 \pm 1.70\times10^2$,

$3.60\times10^2 \pm 0.60\times10^2$, $5.90\times10 \pm 0.90\times10$ and $4.80\times10 \pm 0.60\times10$ at 0, 8, 24 and 36 h, 2, 3, 5 and 7 dafter inoculation, respectively.

While, kariesh cheese batch inoculated with *E. coli* O26: H11 (10^5 CFU/ml) and *L. rhamnosus* (10^8 CFU/ml)gave counts of $6.80\times10^5 \pm 0.30\times10^4$, $7.10\times10^3 \pm 1.50\times10^2$, $4.10\times10^2 \pm 0.80\times10$ and $3.50\times10 \pm 0.20\times10$at 0, 8, 24 and 36 h after inoculation, respectively, but it failed to be detected at 2, 3, 5 and 7d after inoculation as shown in fig. (4).

Contamination with different degrees with *E. coli* give an indication of fecal contamination, poor sanitary measures adopted during milking, manufacturing, handling and distribution of kariesh cheese **(El-Nahas *et al.*, 2015).**

Also table (3) showed kariesh cheese batch inoculated with *S. aureus* alone gave counts of$9.70\times10^5 \pm 1.50\times10^4$, $2.50\times10^4 \pm 2.10\times10^3$, $8.40\times10^3 \pm 1.20\times10^2$, $4.20\times10^3 \pm 0.90\times10^2$, $3.20\times10^2 \pm 0.80\times10$ and $5.60\times10 \pm 0.60\times10$at 0, 8, 24 and 36 h, 2 and 3 d after inoculation, respectively, but it failed to be detected at 5 and 7 d after inoculation.

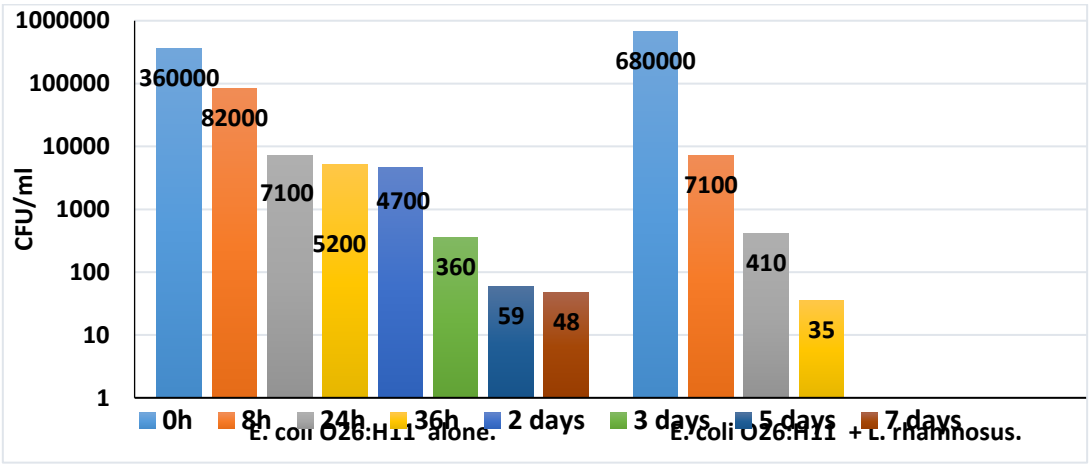

Figure (4): Count reduction and elimination of *E. coli* O26:H11 in kariesh cheese with/without inoculation of *L. rhamnosus*.

Kariesh cheese batch inoculated with *S. aureus*and *L. rhamnosus* (10^8 CFU/ml)gave counts of$6.60\times10^5 \pm 1.30\times10^4$, $7.40\times10^3 \pm 3.20\times10$ and $7.20\times10 \pm 1.30\times10$at 0, 8 and 24 hafter inoculation, respectively, but it failed to be detected at 36 h, 2, 3, 5 and 7d after inoculationas shown in fig. (5).

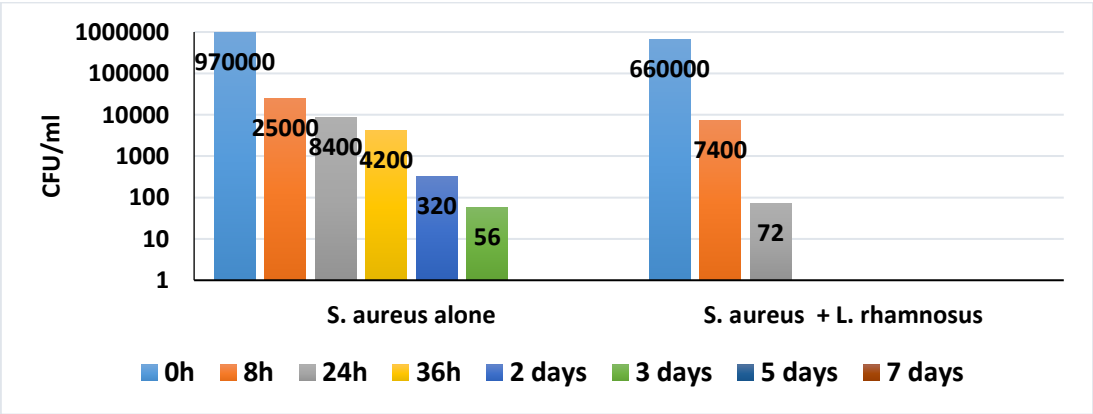

Figure (5): Count reduction and elimination of *S. aureus* in kariesh cheesewith/without inoculation of *L. rhamnosus.*

Production and handling of locally manufactured Egyptian kariesh cheese, is still under-way especially those produced by makers in most villages distributed all-over the country. Therefore, it is contaminated with different types of organisms gaining access to the product from various sources. *S. aureus* is a food born pathogen responsible for an intoxication resulting from the ingestion of food containing preformed heat-stable enterotoxins, usually produced by this microorganism and representing a sanitary risk when levels of specific bacterial counts at least as high as 10^5 CFU/ g or ml of sample are detected. The presence of *S. aureus* in kariesh cheese constitutes a potential public health hazard since many strains of *S. aureus* produce enterotoxins that cause food poisoning if ingested. Neither the absence of *S. aureus* nor the presence of small numbers is complete assurance that a food is safe **(Eid and Eltalawy, 2014)**

Antibacterial effects of probiotics may be due to the production of acetic and lactic acid that lowered the pH of the media**,** and simultaneously produced hydrogen peroxide and bacteriocins that acted as antibiotic agents **(Bezkorovainy, 2001).**

Inspection of fig (6) revealed that the pH values of the incubated kariesh cheese samples decreased (nonsignificant decrease). Nearly similar to those reported by **(Mahmoud*et al.*, 2013)**, while different pH value of kariesh cheese sample 3.80 to 5.00 reported by **(El-Ghaish, 2016)**.This may be due to that probiotic bacteria produce lactic and acetic acids as a metabolic by products which play a complementary role in inhibiting pathogenic and spoilage bacteria (**Tharmaraj, 2004**).

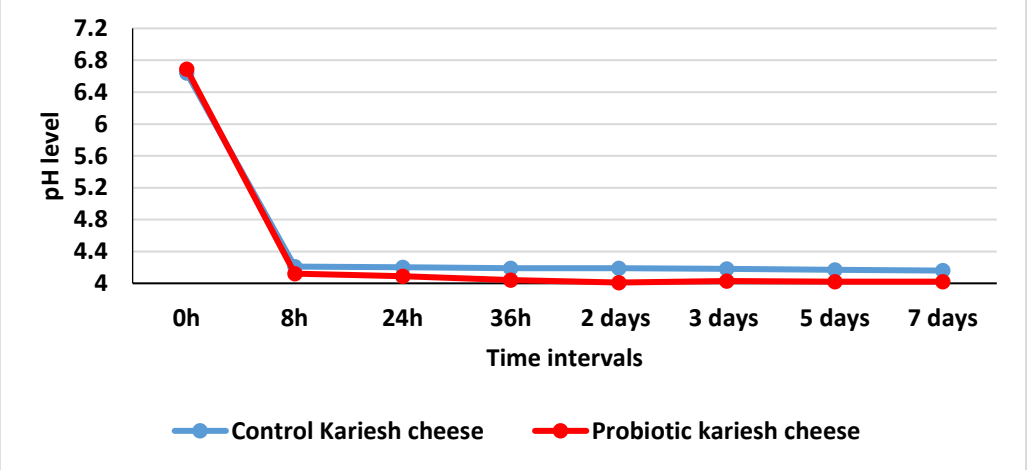

Figure (6): pH level change during storage of control and probiotic kariesh cheesesample

The growth of *L. rhamnosus* during refrigerated storage of Kariesh cheese increased with a peak at 7 days of storage as the highest numbers of bacteria were observed at 7 days of storage **(Mahmoud*et al.*, 2013).**Low pH and high salt concentrations can cause growth inhibition of LAB without reduction of their carbohydrate metabolism leading to formation of acids **(Osman, 2000),** the survival of *l. rhamnosus* detected in different concentration of NaCl % (0, 2, 4, 6 and 8). *L. rhamnosus*resist low pH (2, 2.5 and 3) (**Magdoub*et al.*, 2015).**

In conclusion, these results indicated that the probiotic culture is capable of inhibit the growth of many foodborne pathogens, such as *S. aureus* and *E. coli*. Different mechanisms of action, such as organic acids, bacteriocins and others, seemed to be involved in this antimicrobial activity **(Olasupo *et al.*, 1995).** Bacteriocins are antimicrobial peptides, synthesized and subsequently secreted by gram positive as well as gram negative bacteria **(Berjeaud and Cenatiempo, 2004).**

The action of the bacteriocins may be explained by the interaction with lipoteichoic acids, which are absent in Gram-negative bacteria, and these molecules would play the role of sites needed to produce the bactericidal effect. This last observation is in accordance of other reports and studies which stated that some bacteriocins produced by Gram-positive bacteria, regardless of its source, have a broad spectrum of activity against Grampositive microorganisms than did with Gram- negative **(Savadogo *et al.*, 2004).**

We believe that further understanding of the molecular mechanismof the relationship between microbes (bacteriocin-producers and pathogens) should be explained or at least clarify these findings.

Public health significance:

Probiotics are intended to assist the body's naturally occurring gut microbiota. Some probiotics preparations have been used to prevent diarrhea caused by antibiotics (**Soomro *et al.*, 2002).** Studies have documented probiotics effects on a variety of gastrointestinal and extraintestinal disorders, including inflammatory bowel disease (IBD), irritable bowel syndrome (IBS). Although there is some clinical evidence for the role of probiotics in lowering cholesterol. The strongest clinical evidence for probiotics is related to their use in improving gut health and stimulating immune function **(Tharmarja and Shah, 2004).** A number of studies have found probiotics consumption to be useful in the treatment of many types of diarrhea, including antibiotic associated diarrhea. *Lactobacillus* and *Streptococcus* are the most common probiotics used in commercial fermented and non-fermented dairy products today (**Heller, 2001**).

4. Conclusion

Addition of *L. rhamnosus* to yoghurt and kariesh cheese has an inhibitory effect on *S. aureus* and *E. coli*. Gram positive bacteria (*S. aureus*) were affected more than gram negative bacteria (*E. coli*). The antibacterial activity of *L. rhamnosus* was not lost by long storage at 4°C and remain stable over wide range of pH.

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الملخص العربي

تأثير بكتريا البروبيوتك علي بعض البكتريا المسببة للأمراض فيالزبادي والجبن القريش

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الملخص العربى

أجريت هذه الدراسة لإختبار مقدرة عترة الميكروب النافعةمن البروبيوتك (لاكتوباسيلس راميزيس) لمنع ميكروبات التسمم الغذائي وكذلك التى تعطي مؤشر على التلوث فى الزباديو الجبن القريش مثل(المكور العنقودي الذهبي وايشرشيا كولاي O26:H11)وتمت فى صورة ثلاث تجارب الأولى منها كانت بإستخدام طريقة (agar well diffusion) واسفرت النتائج عن متوسط ثلاث مرات متكرره و أوضحت أن العترة المستخدمة تمنع الميكروبات المستخدمة في تلك التجربة و لكن التأثير الأقوى كان على المكور العنقودي الذهبي و يليه إيشرشيا كولاي O26:H11 أما التجربة الثانية و الثالثة استهدفت منع نمو ميكروبات التسمم الغذائي في وجود البروبيوتك لالكتوباسيلس راميزس أثناء عملية تخمر الزبادي مع التحكم في الأس الهيدروجيني للزبادي عند إضافة البادئ وأثناء تصنيع الجبن القريش. ووجد أن العد لكل من ميكروبات المكور العنقودي الذهبي وميكروب الإيشرشياكولاي O26:H11 بعينات الزبادي المختبرة إختلف عن عدهم في وجود البروبيوتك لالكتوباسيلس راميزس و ذلك يرجع للمواد المضادة للبكتيريا الصادرة عنها. وقد تلاحظ من خلال تلك النتائج أن الوقت المستغرق في التخلص من ميكروبات المكور العنقودي الذهبي وايشرشيا كولاي O26:H11 في عينات الزبادي و الجبن القريش كان أقصر في وجود البروبيوتك لالكتوباسيلس راميزس و تلك النتائج توضح أنها لها القدرة على تعطيل نمو تلك الميكروبات مما يؤكد التأثير المضاد للبروبيوتك ضد تلك الميكروبات عند حقنهمع البادئ بعينات الزبادي و الجبن القريش أثناء التصنيع حيث أنها تعتبر طريقة طبيعية و صحية للحفاظ على الأطعمة و تحسين جودة و صحة و سلامة الغذاء .و قد تم مناقشة خطورة تلك الميكروبات على صحة الإنسان وعلى الناحية الإقتصادية و كذلك طرق التحكم و التخلص منها من أجل تحسين جودة منتجاتالزباديو الجبنة القريش و حماية المستهلك من الإصابةوالعدوى.