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DOI 10.26873/SVR-804-2019 **Original Research Article**

PIVOTAL ROLE OF *Lactobacillus* STRAINS IN IMPROVEMENT OF SOFT CHEESE QUALITY AND INHIBITING THE GROWTH OF HARMFUL AND DANGEROUS BACTERIAL PATHOGENS

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Abstract: This study was carried out to determine the effect of addition of *Lactobacillus* bacteria to improve the soft cheese quality and inhibiting the microbial growth on cheese. *Lactobacillus acidophilus DSM 20079* and *Lactobacillus casei* ss. *casei DSM 20011* were studied *in vitro* for their probiotic properties through their antimicrobial activity. These strains were able to inhibit the growth of *Escherichia coli* O_{157} ATCC51659, *Staphylococcus aureus* and *Bacillus cereus*. Low salt soft cheese was manufactured, with acceptable organoleptic characters and prolonged shelf-life by using *L. acidophilus* and *L. casei* as bio preservative. The results cleared that, the addition of *L. acidophilus* or *L. casei* to the soft cheese improved the quality of the soft cheese through improvement of organoleptic characters of soft cheese such as appearance, body texture, flavour and extended soft cheese shelf-life through microbiological characteristics of manufactured cheese. The effect of the *L. acidophilus* is higher than the effect of *L. casei* than the control group. So we can use these strains as food preservative.

Key words: *Lactobacillus* spp.; cheese making; antibacterial activity

Introduction

Cheese is one of the essential milk products for human feed because it contains most of nutrients required for growth and body health (1). It considered as source of microbial contamination. It is commonly made from non pasteurized milk of poor bacteriological quality, under unhygienic conditions and final product kept uncovered. Thus, there is high threat of contamination with several pathogenic and spoilage microorganisms. Harmful pathogenic bacteria such as *Coliform*, *Escherichia coli*, *Staphylococcus* and *Salmonella* as well as yeasts and molds were recognized in Karish cheese which factory-made with traditional method (2, 3). These harmful microbes cause inflammation and allergic diseases (3). Consumers need natural and chemical-free cheese. Therefore, it became urgent to search for alternative methods for cheese bio-preservation.

Lactobacillus is energetic for recent food technologies, because of their probiotic potential to replace antibiotics (4). Lactic acid bacteria used as a starter for cheese conservation, improving the organoleptic properties, source of fermentation industry and control of food borne

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microbes (5, 6, 7). It enhanced the immune system and produce bacteriocins, organic acids, H_2O_2 as antimicrobial compounds as well as short chain fatty acids (8, 9).

According to our knowledge not enough research were conducted on soft cheese biopreservation in Egypt, therefore, the aim of this research was to study the antimicrobial activity of *Lactobacillus* strains against harmful bacteria such as *E. coli* O_{157},*S. aureus* and *B. cereus* as well as improving the hygienic quality of soft cheese.

Materials and methods

Bacterial Culture

Identified *Lactobacillus acidophilus DSM* 20079 and *Lactobacillus casei* ss. *casei DSM 20011* from Cairo-MIRCEN, Faculty of Agriculture, Ain-Shams University, Cairo, Egypt. Bacteria were cultured on MRS broth (9ml) media then, kept at 37°C for 24hours. After that, they were cultured in sterile skim-milk and incubated at 37°C for 24 hours to activate the bacterial strains and to increase the bacterial number to the target probiotic dose (10^7Colony forming unit/g) for soft cheese manufacture.

Antimicrobial activity of Lactobacillus strains

Antibacterial activity of *Lactobacillus* strains was evaluated by agar well diffusion assay **(10).**Culture media (15 ml), melted and tempered at 45ºC, were inoculated with *E. coli O$_{157}$,S. aureus* and *B. cereus* at 10^6 CFU/ml then poured into Petri dishes that had wells which made by cork borer. Wells were filled with 100 µl of 48 hours-culture-supernatants of the *Lactobacillus* strains, then, incubated at 37 ºC for 24 hours. The bacterial activity seen by appearance of inhibition zone which measured by milli-meter ruler.

Cheese manufacturing

Soft cheese was made as by Mehanna and Rashed 1990 and El-Sheikh *et al.* 2001 (11, 12) with slight modification. Pasteurized half fat milk (12L, 1.5% fat,8.5% SNF) were heated to 40˚C then, the total solid was standardized to 14% by adding 4% skimmed milk powder(<1.25% fat,< 32% protein and >53% lactose), Nacl 3% and CaCl2 0.02% also were added. The bulk volume of milk was divided into 3 groups (4 Kg each) after mixing and inoculated by the activated starter cultures the first group was considered as control, the second was inoculated with *L. acidophilus* culture and the third was inoculated with *L. casei*, then, 0.3g rennet was added to each group and incubated at 37˚C until curd formation, the curd was kept to drain in previously sterilized stainless steal frames lined with cheese cloth. The produced cheese and their whey were packaged in pre-sterilized bottles and kept at4˚C. The cheese was tested fresh (zero time) and at equal intervals of 7 days till the spoilage was appeared. The experiment was repeated in triplicates and average results for each group were tabulated.

Cheese characters evaluation

Organoleptic examination

Cheese samples were tested for appearance, texture, body, and flavor (13). The identical samples were labeled using random numbers and presented to the judges in random manner by 10 examiners.

Chemical examination

Cheese samples were tested for pH (14).

Microbiological examination

Samples were homogenized using sodium citrate (2%) and 10 fold serial dilutions were made (15). Then, they were tested for total *Coliform* count (15); *Lactobacilli* count (16); as well as mold and yeast count (17).

Statistical analysis

The statistical analysis was done using analysis of variance (ANOVA) to compare between the parameters studied among different treatments. Chi^2-test was used also, to determine the incidences of bacterial isolates between different treatments (18).

Results

Antibacterial effect of Lactobacillus strains

Lactobacillus acidophilus and *Lactobacillus casei* ss c*asei* inhibited the growth of harmful bacteria such as *Echerichia coli*O_{157}, *Staphylococcus aureus* and *Bacillus cereus* (Table 1,Fig. 1).

Positive impact of lactobacilli on soft cheese quality

Our results presented in table (2) cleared that, the *Lactobacillus acidophilus* and *Lactobacillus casei* improved the average appearance of the soft cheese and its body texture than the control group and the *Lactobacillus acidophilus* decreased the spoilage followed by the *Lactobacillus casei* than the control group. The results also, cleared that, the cheese still preserved its flavour in case of *Lactobacillus acidophilus* till 32 day from its storage and 30 day in case of *Lactobacillus casei* and in the control group the flavour still present till 14 day of preservation.

The overall score due to addition of *Lactobacillus acidophilus* and *casei* improved the score of cheese especially in *Lactobacillus acidophilus* followed by *Lactobacillus casei* than the control group.

Effect of Lactobacillus bacteria on pH level of soft cheese

The observed results in table (3), exhibited a significant differences ($P < 0.05$) on the effect of lactobacillus bacteria on pH level of soft cheese. *Lactobacillus acidophilus* improved the pH level till 30day of cheese storage, followed by *Lactobacillus casei* and both of them improved the pH level than the control group.

Effect of Lactobacillus level on the bacteriologic aspect of the soft cheese

Our results observed in table (4) cleared that, the level of *Lactobacillus acidophilus* increased gradually by storage time of soft cheese and reached its maximum level at 21 day of storage. By increasing the level of *Lactobacillus acidophilus* and *Lactobacillus casei* causes decreasing the level of coliforms bacterial growth but the effect of *Lactobacillus acidophilus* greater than the effect of *Lactobacillus casei*.

Effect of lactobacillus bacteria on mycological aspect of the soft cheese

Our results observed in Table (5), cleared that, there is a significant different of the effect of lactobacillus bacteria ($P < 0.05$) on the level of mould and yeast count. The results cleared that, the increasing level of *Lactobacillus* associated with decreasing level of moulds and yeast than the control group. The effect of *Lactobacillus acidophilus* higher than the effect of *Lactobacillus casei* in prevention of the growth of moulds and yeast.

Table 1: Inhibition zones (mm) caused by *Lactobacillus* strains in agar diffusion assay (n = 5)

Lactobacillus Species	(Diameter of inhibition zones in mm)		
	Escherichia coli O_{157}	*Bacillus cereus*	*Staphylococcus aureus*
Lactobacillus casei	$2.6±0.20^{B}$	$2.9±0.10^{A}$	$2.75±0.50^{AB}$
Lactobacillus acidophilus	$2.9±0.3^{A}$	$2.57±0.50^{B}$	$2.55±0.20^{B}$
Control	0	0	0

Means within the same row of different litters are significantly different at ($P < 0.05$)

Table 2: Effect of *Lactobacillus* on organoleptic characteristics of soft cheese.

Storage time	Average Appearance (10)			Body texture (60)			Flavor (30)			Overall score (100)		
	Control	*Lactobacillus casei*	*Lactobacillus acidophilic*	Control	*Lactobacillus casei*	*Lactobacillus acidophilic*	Control	*Lactobacillus casei*	*Lactobacillus acidophilus*	Control	*Lactobacillus casei*	*Lactobacillus acidophilus*
Zero time **	7	9	8	56	58	57	28	29	29	91	96	94
7 days	6	8	7.5	55	57	56.5	26	28	28	87	93	92
14 days	5	7	7	45	55	54	19	26	26	69	88	87
21 days	S	5	5	S	50	53	S	20	20	S	75	78
30 days	S	S	4	S	S	52	S	S	19	S	S	75
32 days	S	S	S	S	S	S	S	S	S	S	S	S
Chi^2	10.55**			11.22**			12.45**			9.24**		

S = spoiled ** = Significant at ($P < 0.01$)

Table 3: Effect of *Lactobacillus* on pH of the soft cheese**.**

Storage time	Average pH		
	Control	*Lactobacillus casei*	*Lactobacillus acidophilus*
Zero time	5.54 ± 0.04^A	$5,78 \pm 0.02^A$	5.64 ± 0.04^A
7 days	5.54 ± 0.05^A	5.78 ± 0.01^A	5.64 ± 0.04^A
14 days	5.45 ± 0.05^A	5.76 ± 0.03^A	5.54 ± 0.05^A
21 days	S	5.64 ± 0.04^B	5.50 ± 0.05^B
30 days	S	S	5.35 ± 0.03^B
32 days	S	S	S

S = spoiled. Means within the same row with different litters are significantly different at ($P < 0.05$)

Table 4: Effect of *Lactobacillus* on bacteriological aspect of the examined samples of soft cheese

Storage time	Average coliforms count(cfu/g)			Average Lactobacilli count (cfu/g)	
	Control	*Lactobacillus casei*	*Lactobacillus acidophilus*	*Lactobacillus casei*	*Lactobacillus acidophilus*
Zero time**	$9.9x10^6 \pm 9.0$ $X10^3$ A	$4.5x10^6 \pm 1.50$ $X10^3$C	$2.5x10^5 \pm 1.0$ $X10^3$ D	$1.4x10^7 \pm 3.0$ $X10^3$ B	$1.3x10^7 \pm 3.0$ $X10^3$ B
7 days	$1.3x10^8 \pm 0.50X10^3$A	$2x10^7 \pm$ 2.50 $X10^3$ B	$3x10^5 \pm 1.0$ $X10^3$ C	$2x10^7 \pm 3.0$ $X10^3$ B	$1.8x10^7 \pm 3.0$ $X10^3$ B
14 days	$1.5x10^8 \pm 4.0$ $X10^3$ A	$4x10^6 \pm 3.0$ $X10^3$ C	$1x10^5 \pm 1.0$ $X10^3$ D	$2.8x10^7 \pm 3.0$ $X10^3$ B	$6.13x10^7 \pm 2.0$ $X10^3$ B
21 days	S	$1x10^3 \pm 1.0$ $X10^3$ D	$11x10^2 \pm 1.0$ $X10^3$ D	$1.1x10^7 \pm 1.0$ $X10^3$ D	$5.2x10^7 \pm 1.0$ $X10^3$ D
30 days	S	S	$18x10^2 \pm 1.0$ $X10^3$ B	S	$2.21x10^7 \pm$A1.0 $X10^3$
32days	S	S	S	S	S

S= spoiled. Means within the same row of different litters are significantly different at $P < 0.05$

Table 5: Effect of *Lactobacillus* on mycological aspect of the soft cheese

Storage time	Average mould count (cfu/g)			Average yeast count (cfu/g)		
	Control	*Lactobacilus casei*	*Lactobacillus acidophilus*	Control	*Lactobacillus casei*	*Lactobacillus acidophilus*
Zero time	<10D	<10D	<10D	$1.3x10^7$A	$4.80x10^6$B	$1.50x10^5$ C
7 days	<10D	<10D	<10D	$1x10^8$A	$2.5x10^7$B	$2.20x10^6$C
14 days	$2x10^1$D	<10D	<10D	$1.3x10^8$A	$2.40x10^7$B	$1.10x10^6$C
21 days	S	$1.50x10^1$C	<10D	S	$2.17x10^4$A	$1.18x10^4$B
30 days	S	S	$2x10^1$B	S	S	$1.06x10^3$A
32 days	S	S	S	S	S	S

S= spoiled, Means within the same row of different litters are significantly different at ($P < 0.05$)

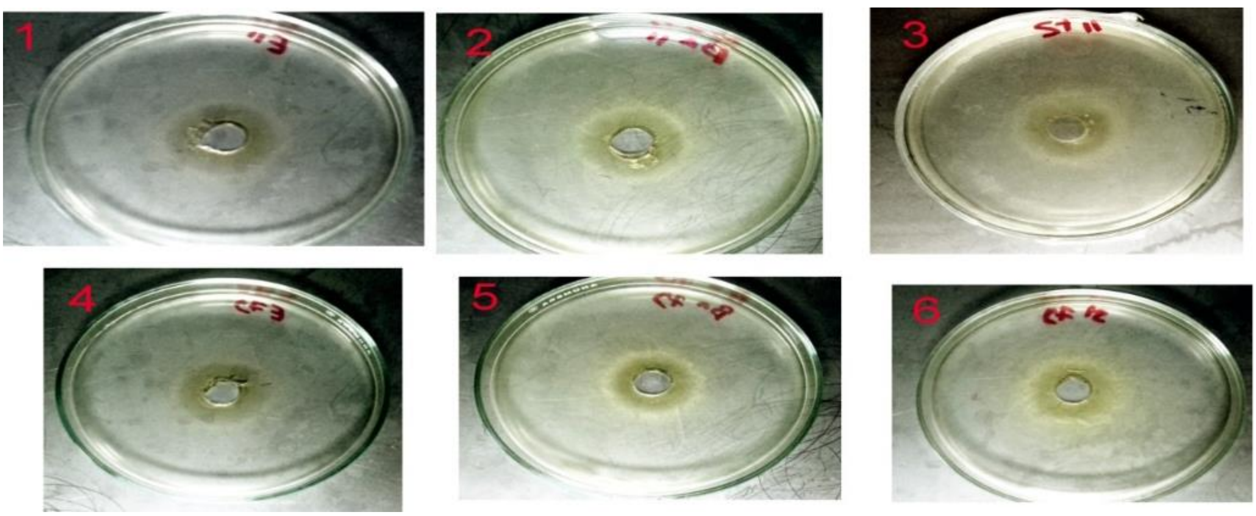

Figure 1: Antibacterial activity of *Lactobacillus casei* ss. *casei DSM 20011* against *(1) Escherichia coli* O_{157} *ATCC51659 (2) Bacillus cereus (3) Staphylococcus aureus* determined by appearance of inhibition zone(mm). Antibacterial activity of *Lactobacillus acidophilus DSM 20079* against *(4) Escherichia coli* O_{157} ATCC51659 *(5) Bacillus cereus (6) Staphylococcus aureus* determined by appearance of inhibition zone (mm)

Discussion

Influence of *Lactobacilli* on cheese quality showed that both of *Lactobacillus* strains were able to improve cheese appearance, body texture, flavour, than the control group. *Lactobacillus acidophilus* decreased the spoilage incidence than *Lactobacillus casei* than the control. These results attributed to the increasing pH level which prevents the cheese spoilage with improvement of sensory characters. *L. acidophilus* improved the pH level till 30 days of cheese storage, followed by *L. casei* as compared to control group. The effect of *Lactobacilli* level on the bacteriologic aspect of the soft cheese cleared that, the level of *L. acidophilus* increased gradually by storage time of soft cheese and reached to its maximum level at 21 day of storage. The obtained data from microbial quality assessment of collected soft cheese samples indicated that, their inferior hygienic quality with a great chance of being a cause of food-borne disease. According to the Egyptian Standard ES 1008-2000; 50 % of cheese samples are not agreed due to the high counts of coliform as fecal contamination indicator mostly in cheese variety (19).

As a result of increasing the level of *L. acidophilus* and *L. casei*, the level of *Coliforms* bacterial growth was decreased however the effect of *L. acidophilus* greater than the effect of *L. casei*. These positive results attributed to the *Lactobacillus* ability to improve the pH level which prevents the growth of *coliforms* in soft cheese.

High yeast count regularly indicates disused hygienic measures during production and management (2). The effect of *Lactobacillus* bacteria on mycological side of soft cheese proved that, there is a significant difference effect of the bacteria ($P < 0.1$) on yeast and moulds count. The increasing level of *Lactobacilli* correlated with decreasing level of yeast and moulds compared with control group. Interestingly, the effect of *L. acidophilus* higher than *L. casei*e effect in prevention of moulds and yeast growth. *Lactobacilli* as biotherapeutic agents serve as natural food preservative through the antimicrobial bacterial activity (3). Both *Lactobacillus* strains were able to inhibit the growth of *Escherichia coli* O_{157}, *Staphylococcus aureus* and *Bacillus cereus* as is seen by appearance of inhibition zone. The obtained results were in supported with that results in which proved the antagonistic activity of *Lactobacillus* against *Staphylococcus* spp. and *Coliform* sp. (20, 21).

We were able to produce soft cheese with advanced procedure and reached the optimum hygienic conditions. Increased *Lactobacillus* counts might be relied on the absence of nutrients competition between *Lactobacillus* and other microorganisms, particularly in the individual culture treatments. *Lactobacillus* spp. compete with other microbes their metabolic end products (22). Molds absence as a result of treatments till the 21th day of cold storage, may translate the antimicrobial effect of those two promising strains which achieve the main aim of this study in better microbial quality and extended shelf life of produced cheese. These results agreed with (23) who stated that; *Lactobacillus* isolated from fermented foods produce organic acids and other antimicrobial agents, which are responsible for quality and palatability.

Analysis of chemical and texture analyses, where less protein/ dry matter % decreased hardness and compacted structure of the new products causing softness. Keeping organoleptic characteristics similar to conventional soft cheese with higher hygienic quality is encouraging. These results agreed with what reported that *Lactobacilli* are members of autochthonous non-starter lactic acid (24). The relation with the chemical properties where compact structure was a result of relatively higher protein/ dry matter % of control. The higher moisture content, the more soft cheese that coats the mouth during eating (25). Such characteristics improvements could increase consumer demand to the new cheese product.

Conclusion

It can be concluded that this research study cleared that, the two promising strains of *Lactobacillus acidophilus* and *Lactobacillus casei* ss. *casei* were able to inhibit the growth of harmful food pathogenic bacteria such as *Escherichia coli* O_{157}, *Staphylococcus aureus* and *Bacillus cereus*. This perhaps the first study which conducted on *Escherichia coli* O_{157} as one of the dangerous and fetal human bacteria in Egypt. In addition to that both of *Lactobacillus* strains improved the quality of the soft cheese through improving the pH value, improvement of organoleptic characters such as appearance, body texture and flavour as well as improving the total score level of the soft cheese microbiological characters.

The positive effect of the *Lactobacillus acidophilus* is higher than *Lactobacillus casei* effect than the control group.

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Conflict of interest

The authors declare that they have no conflict of interest.

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