



INHIBITION OF *STAPHYLOCOCCUS AUREUS* BY LACTIC ACID BACTERIA AND / OR *BIFIDOBACTERIUM LACTIS* DURING MILK FERMENTATION AND STORAGE

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ABSTRACT

Survival and inhibition of *Staphylococcus aureus* by the lactic acid bacteria (LAB) starter culture (*Streptococcus thermophilus* and *Lactobacillus delbrückii* subsp. *bulgaricus*) and/ or probiotic bacteria *Bifidobacterium lactis* during milk fermentation to yoghurt and storage up to 12 days was studied. Adding *S. aureus* (initial count log 6.64/ ml) with LAB (initial count log 6.8/ ml) in milk during yoghurt processing and storage resulted in no significant change in the counts of both *S. aureus* and LAB during fermentation period of 4 hrs at 45° C. A steady decrease in *S. aureus* count during storage at 25° C and 4° C was observed reaching a complete (100 %) inhibition after 9 and 12 days, respectively, with no significant increase in LAB count. Adding *S. aureus* (initial count log 6.62/ ml) with *B. lactis* (initial count log 6.83/ ml) in milk for 4 hr at 45° C, no significant changes in the counts of both bacteria were found. After storage at 25° C and at 4° C a sharp decline in the *S. aureus* count with a 100 % inhibition after 6 and 9 days with approximately two log and one log increase in *B. lactis* counts consecutively. In general similar result was observed when adding *S. aureus* together with LAB and *B. lactis* in milk during fermentation and storage. pH values decreased during milk fermentation and storage from initially 6.55-6.64 to around 4 in most milk samples.

The results of this study show that *S. aureus* was completely inhibited by LAB and/or *B. lactis* after milk fermentation to yoghurt and storage at room temperature and refrigeration for 6-9 days. It is therefore recommended to add the probiotic *B. lactis* with LAB to milk for yoghurt processing.

Keywords: *S. aureus*, Lactic acid bacteria, *Bifidobacterium lactis*, inhibition, milk fermentation.

INTRODUCTION

Staphylococcal food poisoning is one of the most common types of food borne disease worldwide. It is intoxication, resulting from the ingestion of food containing one or more preformed *Staphylococcal* enterotoxins (Cliver and Riemann, 2002). Among *Staphylococci* species, *S. aureus* is the most important species that causes food borne diseases. *S. aureus* is pathogenic, Gram positive bacterium and the causative agent of a wide panel of infections ranging from superficial lesions to life threatening septicemia. The natural ecological niches of the species are the nasal cavity and skin of the warm blooded animals. Because of its importance and highly prevalence, there is an increasing interest in bacterial interactions and in understanding of the mechanisms by which the inhibition of *S. aureus* by other bacterial species may occur (Charlier *et al.*, 2009). Lactic acid bacteria (LAB) have been used in food fermentation for centuries. LAB plays an important role in food industry, because they contribute to the flavor, texture, and nutritional values of the food products. The interest in application of LAB and their metabolites in food industry mainly related to the prevention of food spoilage and extending shelf life of foods and the capacity to inhibit the growth of pathogenic microorganisms (Topisirovic *et al.*, 2006). The inhibition of *S. aureus* by LAB has been widely studied. Abee *et al.*, (1995) reported that LAB, as starter culture are routinely employed in the fermented food and food products to inhibit the growth of spoilage and pathogenic bacteria in fermented meat, dairy and other groups of food products. *Bifidobacteria* are natural component of the gastrointestinal micro-biota, and are generally recognized as probiotics “live microorganisms which when administered in adequate amounts confer a health benefit to the host” (Martins *et al.*, 2010). Several health promoting properties of *Bifidobacterium* have been proposed, including inhibition of pathogenic bacteria in the intestine. One of the proposed mechanisms of inhibition is through production of antimicrobial substances. Antimicrobial components produced by *Bifidobacterium* include

organic acids, hydrogen peroxide, carbon dioxide, diacetyl, and bacteriocins (**Lahtinen et al., 2007**).

This study is aimed to investigate the survival and inhibition of the pathogenic bacteria *S. aureus* with or without the addition of LAB and/or *B. lactis* as probiotic in fresh cow's milk during fermentation process to yoghurt and during prolonged storage at room temperature and refrigeration.

MATERIAL AND METHODS

Isolation and characterization of *S. aureus*

Wild enterotoxigenic *S. aureus* isolates were isolated from different food samples (white cheese, cow's milk, chicken, lamb and beef meat) purchased from local markets in Amman, Jordan. The isolates were subjected to purification and characterization tests including coagulase, catalase and DNase for *S. aureus* pathogenicity according to (**AOAC, 1995; Oxoid manual, 2006; Bergey's manual, 1986**). Fifty gm from each food sample was weighed, and added to 450 ml sterilized peptone water in a sterilized polyethylene bag and homogenized in a stomacher for two minutes at high speed. This 1:10 dilution was used to prepare further serial dilutions up to approximately 10^{-6} . From each dilution 1 ml was inoculated into tubes containing sterilized trypticase soya broth with 10 % sodium chloride and 1 % sodium pyruvate, and then incubated for 48 hour at 35-37° C. From each growth positive tube one loopful was transferred and streaked to Baird Parker agar (BPA) plates enriched with egg yolk tellurite emulsion (50 ml emulsion/ Liter BPA) to obtain isolated colonies. BPA plates were incubated for 48 hr at 35-37° C. Each well defined *S. aureus* colonies were subjected to the above confirmation tests of pathogenicity.

Inocula preparation and Inoculation

Inocula of approximately 10^9 / ml of each bacterium were added to one liter of fresh milk resulting in an initial count of approximately 10^6 / ml. Quadruplicate test tubes containing sterilized BHI broth with a pure colony of *S. aureus* each were incubated for 24 hr at 37° C. Serial dilutions of the culture up to 10^{-9} were made and one ml of each dilution was spread over triplicate solidified BPA and incubated for 48 hr at 37° C. The results indicated that the \log_{10} numbers were 9.48, 9.3, 9.7 and 9.85 with an average of 9.6 / ml of BHI broth. This

procedure of determining the log number of *S. aureus* was used in all progressive experimentation. Preparation of approximately 10^9 / ml inocula of *B. lactis* were obtained by mixing 10 gm of *B. lactis* culture powder (each gram contains log 11 viable cells) into 90 ml sterilized 0.1 peptone water to obtain 10^{-1} dilution. Further dilutions are proceeded to obtain 10^9 viable cells/ ml to be used in experimentation. Approximately 10^6 / ml viable cells of yoghurt LAB starter culture (*S. thermophilus* and *L. delbrukii* subsp. *bulgaricus*) obtained by adding two sachet of mixture culture of LAB to 500 liter pasteurized milk.

Cell counts

Viable cell counts of samples during fermentation and storage period for each bacterium were determined with spread plate method using selective medium for each as follows:

For the determination of *S. aureus* viable cell counts, one ml of an appropriate dilution of each sample was aseptically transferred onto triplicate plates of BPA, and equitably distributed (0.4 ml, 0.3 ml and 0.3 ml). Spread inocula over the surface of media using sterile, bent glass streaking rods, avoiding extreme edges of the plate. Retain plates in upright position until inocula are absorbed by medium. Invert plates and incubate for 45-48 hr at 35-37° C. The number of colonies on the appropriate triplicate plates is multiplied by dilution factor to get the cell count of *S. aureus*/ gm of test sample (AOAC, 1995).

B. lactis was enumerated by aseptically transferring 0.1 ml of an appropriate dilution to each of duplicate MRS agar plates, supplemented with 1% raffinose as source of energy, 0.05% lithium chloride to suppress *L. delbrukii* subsp. *bulgaricus* growth, 0.05% L-cysteine hydrochloride which helps in providing anaerobic condition for *B. lactis* growth. Inocula were spread over the surface of media using sterile bent glass streaking rods, avoiding extreme edges of the plate. Plates were incubated in anaerobic jars for 72 hr at 45° C. This temperature suppresses the growth *S. thermophilus* (Tobasco et al., 2007). *B. lactis* count in samples is calculated similar to that of *S. aureus*.

S. thermophilus was enumerated by aseptically transferring 0.1 ml of the appropriate dilution to each of duplicate M17 agar plates, supplemented with 19 gm/ L di-sodium glycerophosphate which has sufficient buffering capacity to maintain the pH above 5.7 which enhance the growth of *S. thermophilus* and suppress the growth of *L. delbrukii* subsp. *bulgaricus* (Oxoid manual, 2006). Plates were incubated aerobically for 48 hour at 37° C. typical colonies of white-cream color, 1-1.5 mm in diameter (Camschella et al., 1998; Rybka

and Kailasapathy, 1996) are counted, then the bacterial counts were calculated. *L. delbrukii* subsp. *bulgaricus* was enumerated by aseptically transferring 0.1 ml of the appropriate dilution to each of duplicate MRS agar, supplemented with acetic acid until the pH of the medium reach 5.4 (acidified MRS). Plates were incubated in anaerobic jars for 72 hr at 37° C. Typical colonies which have lenticular often sharp-shaped forms with 1-3 mm in diameter (International Dairy Federation Standard, 117: 1988) were counted and counts/ ml were calculated.

Experimentations

The suitable serial dilutions of each of *S. aureus*, LAB and *B. lactis* prepared containing approximately log 9 viable cells/ ml were inoculated to one liter of fresh pasteurized milk thus the inocula will approximately be log 6/ ml for each of the following treatments:

- | | |
|--|--|
| 1- <i>S. aureus</i> (as control). | 2- <i>S. aureus</i> + LAB. |
| 3- <i>S. aureus</i> + <i>B. lactis</i> . | 4- <i>S. aureus</i> + LAB + <i>B. lactis</i> . |

Initial counts of each bacterium were estimated and all containers were incubated for 4 hr at 45° C. Milk coagulation of each sample was recorded and bacterial counts were estimated. The duplicate samples of each treatment were divided to two batches; one batch is stored at room temperature (20-25° C) and the other stored in refrigerator (4° C). Bacterial counts in log/ ml and pH values of each treatment were monitored and recorded for the survival and inhibition of *S. aureus* after 4 hr, 8hr, 24 hr, 3 days, 6 days, 9 days and 12 days from the time of inoculation.

Statistical Analysis

Statistical analysis of bacterial counts of different growth interaction experiments were performed using GLM procedure of SAS. The model included treatment (growth interaction) as main plot, temperature of storage as subplot and period of storage as sub-subplot in a split-split-plot design. pH was included as a covariate for the bacterial count analysis model. Fisher protected t-test at $p < 0.05$ was also performed for significant effects of means of different factors.

RESULTS AND DISCUSSION

Isolation and characterization of *S. aureus*

S. aureus was isolated from 16 different food samples (white cheese (5), cow's milk (5), chicken (2), lamb (2) and beef meat (2)) purchased from local markets. Colonies of *S. aureus* over BPA after 48 hr incubation at 37° C were found to be typically circular, smooth, convex, moist, 2-3 mm in diameter, gray-black to jet black, frequently with off-white margins, surrounded by opaque zone (precipitate), and frequently with outer clear zone. The identity of *S. aureus* was confirmed by being Gram positive and cocci (arrangement as mono-cocci, di-cocci, tri-cocci and clusters). The ability of *S. aureus* to produce enterotoxins includes tests for catalase, coagulase and DNase enzymes (Table 1). It was found that isolates from all food samples were catalase positive. All isolates from 4 out of 5 white cheese samples were coagulase and DNase positive.

Jay, (2000) reported that 93% of enterotoxigenic strains of *S. aureus* produce coagulase, and 95% produce DNase. **Cliver and Riemann, (2002)** stated that coagulase positive *S. aureus* are able to produce enterotoxins. An isolate of *S. aureus* from white cheese (number 14) was found to be the most active clot forming with rabbit plasma. It is given the symbol (14) which is used in these experiments thereafter.

Table 1 Enzymes confirmation tests* to confirm the ability of *S. aureus* isolated from different food samples to produce enterotoxins.

Isolate number	Food sample	Catalase test	Coagulase test	DNase test
1	Cow's milk	+	-	-
2	Cow's milk	+	-	-
3	Cow's milk	+	-	-
4	Cow's milk	+	-	-
5	Cow's milk	+	-	-
6	Lamb meat	+	-	-
7	Lamb meat	+	-	-
8	beef meat	+	-	-
9	beef meat	+	-	-
10	Chicken meat	+	-	-
11	Chicken meat	+	-	-
12	White cheese	+	++	+
13	White cheese	+	++	+
14	White cheese	+	++++	+
15	White cheese	+	-	-
16	White cheese	+	+++	+

* = Catalase, Coagulase and DNase, + = positive, - = negative.

S. aureus growth in milk

Inocula of approximately $9 \log_{10}$ of *S. aureus* (14) were transferred into each of two plastic containers each containing one liter fresh pasteurized cow's milk and incubated for 4 hr at 45° C. The initial count was calculated to be $6.78 \log_{10}$ / ml with a pH value of 6.64. It was found that no significant increase in *S. aureus* counts ($6.89 \log_{10}$ / ml) during this time with slight decrease in the pH value (6.24). The non increase of the bacterial cells during this period was probably due to the lag phase required for this bacterium before the beginning of cell division. In addition, the higher temperature of incubation (45° C) is not favorable for the growth of this bacterium. After transferring milk sample (1) to room temperature and sample (2) to refrigeration the log number of *S. aureus* was slightly increased ($7.11 \log_{10}$) after 8 hr and to $7.62 \log_{10}$ after 24 hr of storage at room temperature. The count of *S. aureus* decreased after 3 days ($6.82 \log_{10}$), 6 days ($5.7 \log_{10}$), 9 days ($4.49 \log_{10}$) and the lowest was after 12 days ($2.86 \log_{10}$). No significant changes in the count of *S. aureus* in milk during refrigeration storage (7.5 - $7.9 \log_{10}$). This slight increase may be due to the cell multiplication during the transitional period of time after incubation period at 45° C and before reaching refrigeration

or room temperatures. pH values decreased from 6.24 after 4 hr of incubation at 45° C to 4.94 after 8 hr storage reaching a minimum 3.93 after 12 days of storage at room temperature. This decrease in pH value is probably due to the production of lactic acid or other organic acid as a result of the growth of pasteurization resistant bacteria, or due to production of organic acids by *S. aureus* itself as it was reported by (Fujikawa and Morozumi, 2006). This may also be the reason behind the decrease in the count of *S. aureus* in milk during storage.

Growth inhibition of *S. aureus* by yoghurt LAB starter culture in milk

The growth inhibition interaction of *S. aureus* by yoghurt LAB starter culture (*S. thermophilus* and *L. delbrukii* subsp. *bulgaricus*) in fresh pasteurized cow's milk during fermentation process and extended storage up to 12 days in room and refrigeration temperatures is presented in (Figure 1). It was found that the viable cell counts of *S. aureus* (14) slightly decreased from an initial number of 6.62 log₁₀/ ml to 6.36 log₁₀/ ml during fermentation period, and significantly (P<0.05) decrease during storage period with complete inhibition after 9 days of storage at room temperature and 12 days at refrigeration. pH values were also significantly (P<0.05) decreased during fermentation period of 4 hr and slightly decreased during storage period at both room and refrigeration temperatures. LAB starter culture counts were slightly increased (0.5 log₁₀) during fermentation period and significantly (P<0.05) increased (1.5 log₁₀) during storage period at room temperature with no significant changes in their counts during refrigeration storage. These results show that the antagonistic effect of LAB on *S. aureus* (14) was related to the decrease in the pH value from initially 6.64 to 3.93 at day 9 of storage at room temperature and to 4.02 after 12 days at refrigeration. The decrease in pH values was as a result of the breakdown of lactose in milk to lactic acid. The competition on nutrient and possible bacteriocin production by LAB may have also been contributing to the decrease and complete inhibition of the growth of *S. aureus* (14) in fermented milk. Charlier *et al.*, (2009); Le Marc *et al.*, (2009) stated that the inhibition of *S. aureus* by LAB starter culture is related to many antagonistic factors including the decrease in pH as a consequence of lactic acid production, competition for nutrient, production of H₂O₂ and production and accumulation of anti *Staphylococcal* substances such as bacteriocin.

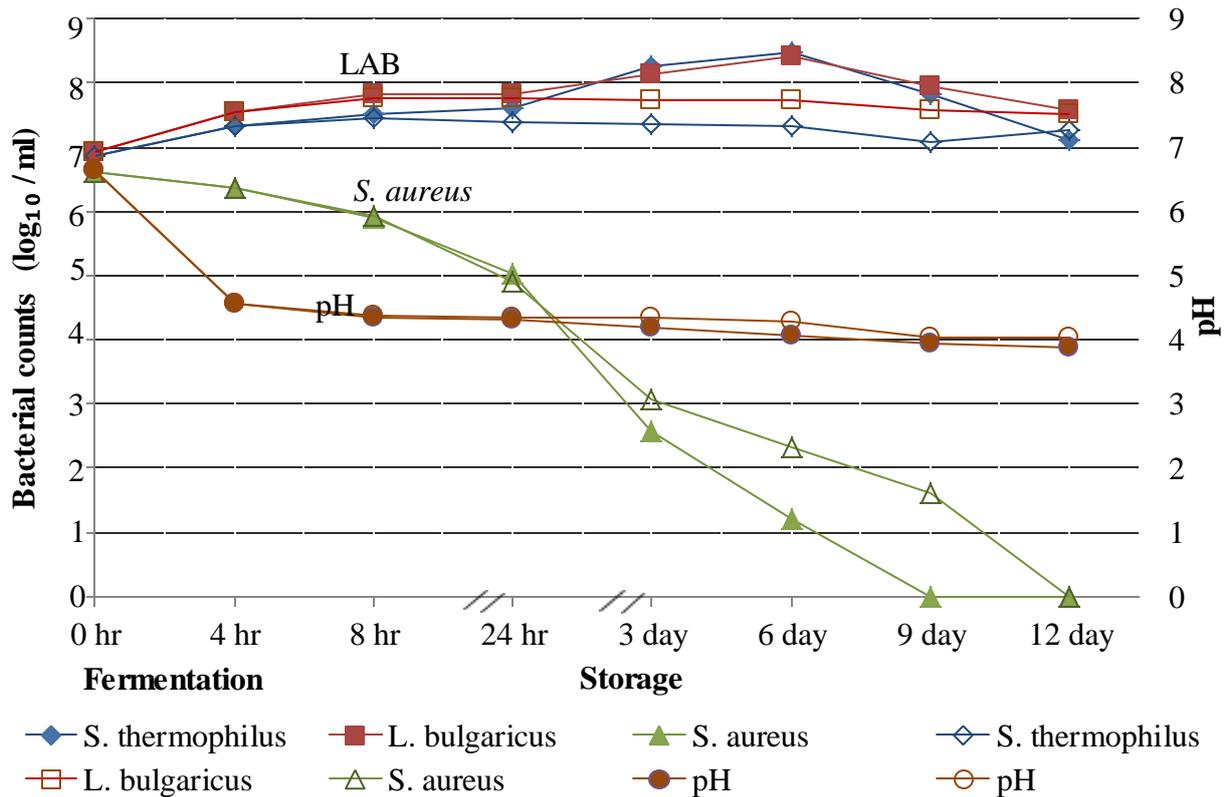


Figure 1 Growth inhibition of *S. aureus* by LAB during milk fermentation and storage at room (full symbols) and refrigeration (empty symbols) temperature with pH values

Growth inhibition of *S. aureus* by *B. lactis* in milk

The results of the experiment of growth interaction between *B. lactis* and *S. aureus* (14) in fresh pasteurized cow's milk during the 4 hr fermentation period and storage for a period of 12 days at room and refrigeration temperatures are presented in (Figure 2). The results show a slight decrease in the counts of *S. aureus* (14) (0.37 log₁₀) and an increase in the growth of *B. lactis* (0.63 log₁₀) with a slight decrease in the pH values (from 6.55 log₁₀/ ml initially to 6.09) after 4 hr fermentation period. During storage at room temperature *S. aureus* (14) count was significantly (P<0.05) decreased to reach 2.9 log₁₀ after 3 days with complete inhibition after 6 days of storage. The growth of *B. lactis* continued to increase in count reaching a maximum of 8.94 log₁₀ after 9 days of storage. pH value was significantly (P<0.05) decreased to its minimum of 4.11. At refrigeration temperature *S. aureus* (14) count also decreased significantly (P<0.05) to a minimum of 2.6 log₁₀ after 6 days of storage with a complete inhibition after 9 days of storage. *B. lactis* count continued to be approximately the same throughout the storage period with no significant changes in the pH value. The result of

this experiment shows an effective antagonistic effect by *B. lactis* against *S. aureus* (14) in both samples stored at room and refrigeration temperatures. The antagonistic effect of *B. lactis* against *S. aureus* may be due to the same reasons as the effects of LAB. **Lahtinen et al., (2007)** reported that the inhibitory effect of *B. lactis* against *S. aureus* is related to hydrogen peroxide formation and possible production of heat stable proteinaceous compounds. The effect of this antagonism on *S. aureus* (14) in refrigeration is relatively less, which is probably due to the slower metabolic activities of *B. lactis*. This experiment shows that in addition to the beneficial human health value of *B. lactis* added to fermented milk products as a probiotic, it is also similar to LAB effective as an antipathogenic *S. aureus* (14).

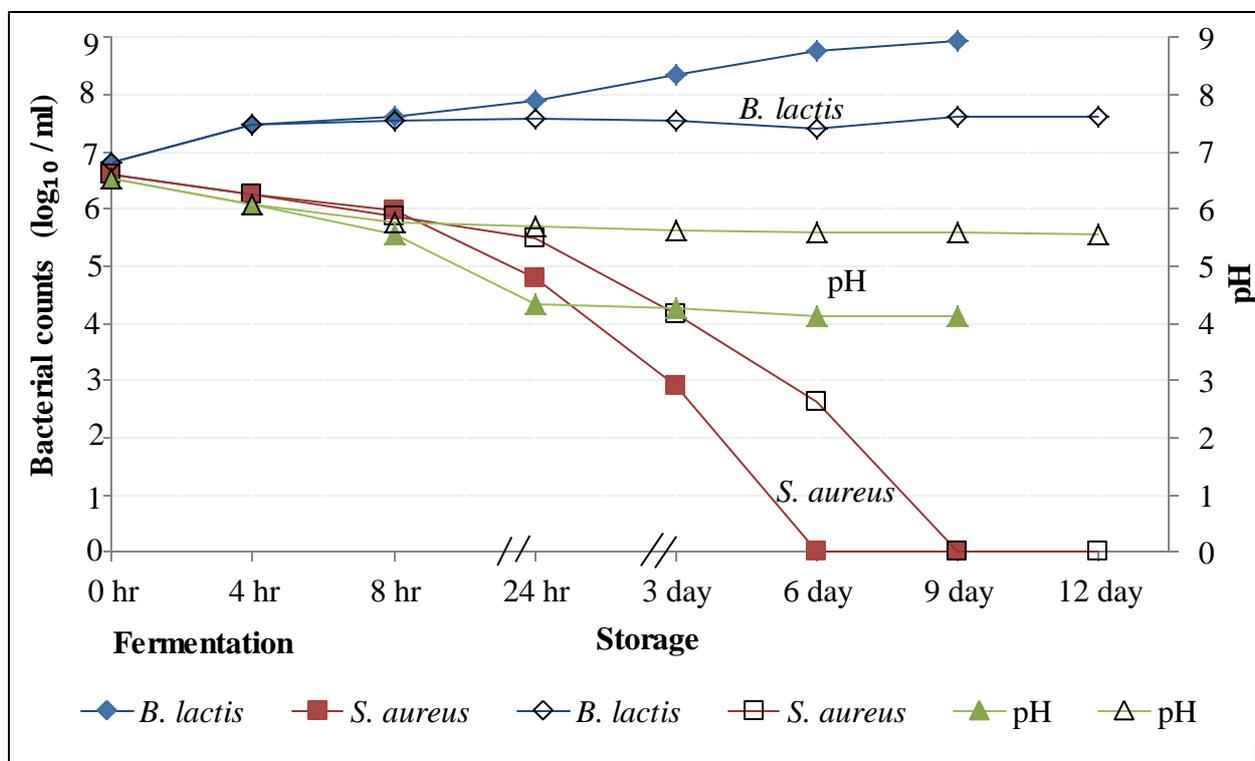


Figure 2 Growth inhibition of *S. aureus* by *B. lactis* during milk fermentation and storage at room (full symbols) and refrigeration (empty symbols) temperature with pH values

Growth inhibition of *S. aureus* by both *B. lactis* and LAB starter culture in milk

The combined growth antagonistic effect of the mixture of LAB and *B. lactis* against *S. aureus* (14) growth during milk fermentation to yoghurt and storage period of 9 days at room and refrigeration temperatures is presented in (Figure 3 & 4). In general the effect of the combined LAB with *B. lactis* against *S. aureus* (14) during milk fermentation and storage periods is similar to the effect of each alone. The combined antagonistic effect of these

bacteria on the growth of *S. aureus* (14) show that the *S. aureus* (14) count was significantly ($P < 0.05$) reduced reaching a complete inhibition after 6 days and 9 days of storage at room and refrigeration temperatures, respectively. In the previous experiments nearly similar result was recorded with *B. lactis* alone, but less antagonistic effect of LAB against *S. aureus* (14) was found.

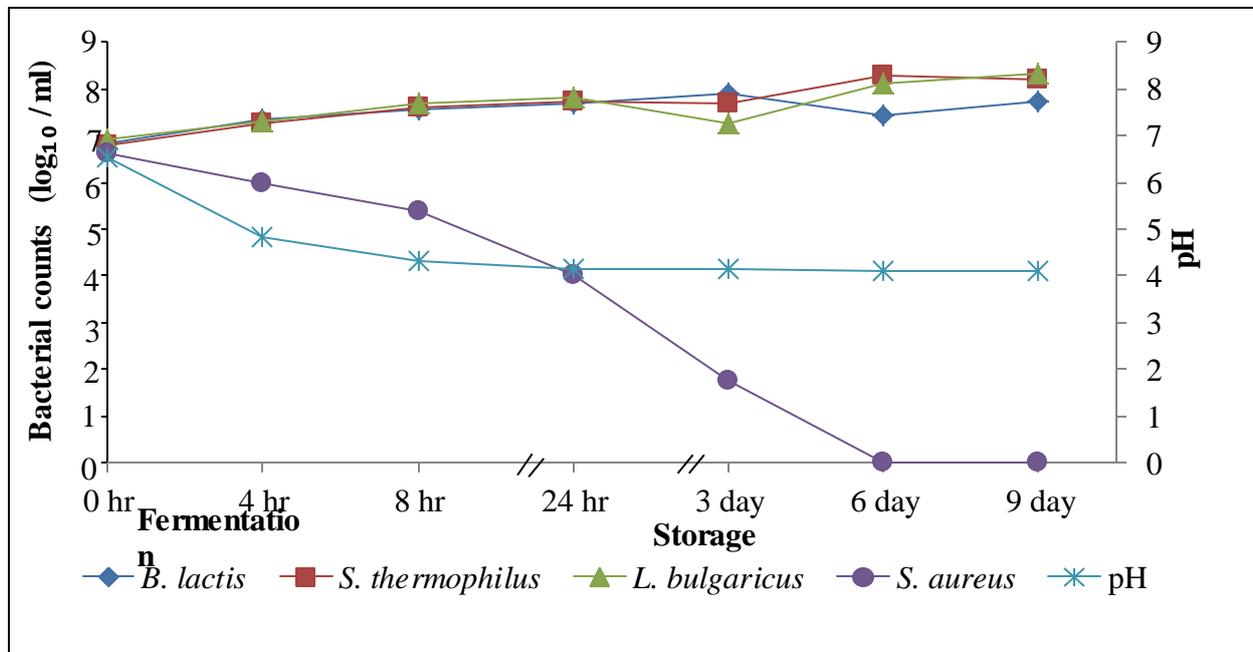


Figure 3 Growth inhibitions of *S. aureus* by LAB with *B. lactis* during milk fermentation and storage at room temperature with pH values

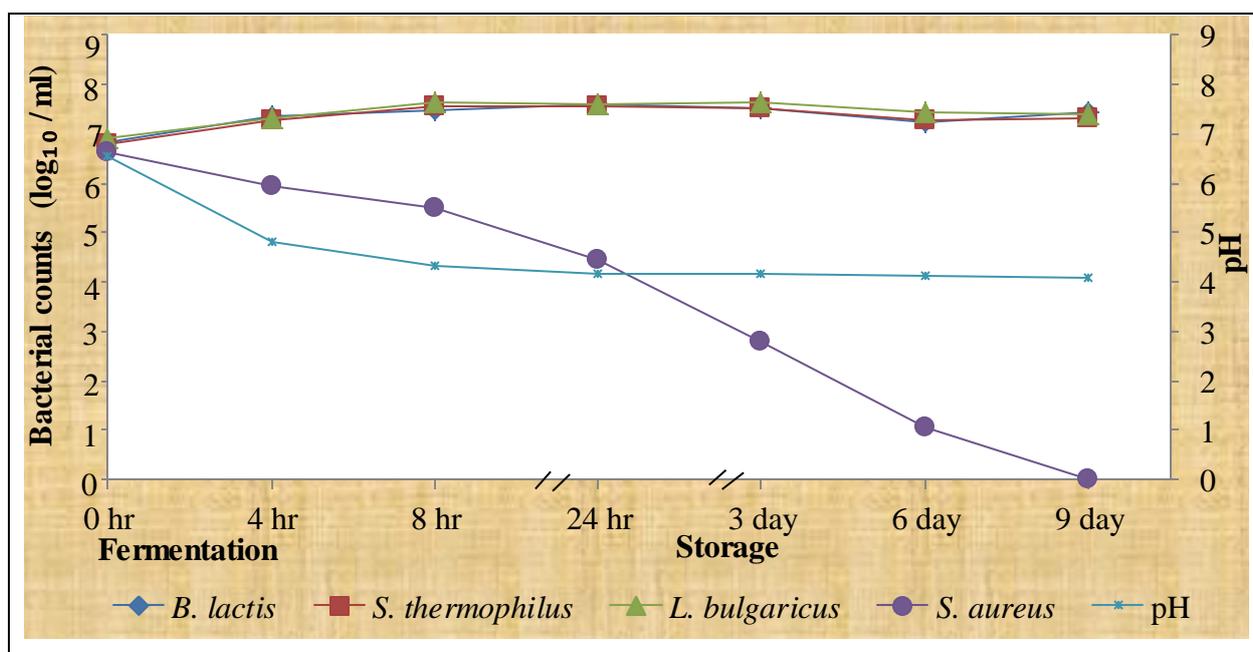


Figure 4 Growth inhibitions of *S. aureus* by *B. lactis* during milk fermentation and storage refrigeration temperature with pH values

Figure (5 & 6) summarizes the growth inhibition of *S. aureus* (14) by *B. lactis*, yoghurt LAB starter culture (*S. thermophilus* and *L. delbrukii* subsp. *bulgaricus*) and the combination of both *B. lactis* and yoghurt LAB starter culture compared to growth of *S. aureus* (14) alone in fresh pasteurized cow's milk during 12 days of storage at room and refrigerator temperatures after an incubation period of 4 hr at 45° C.

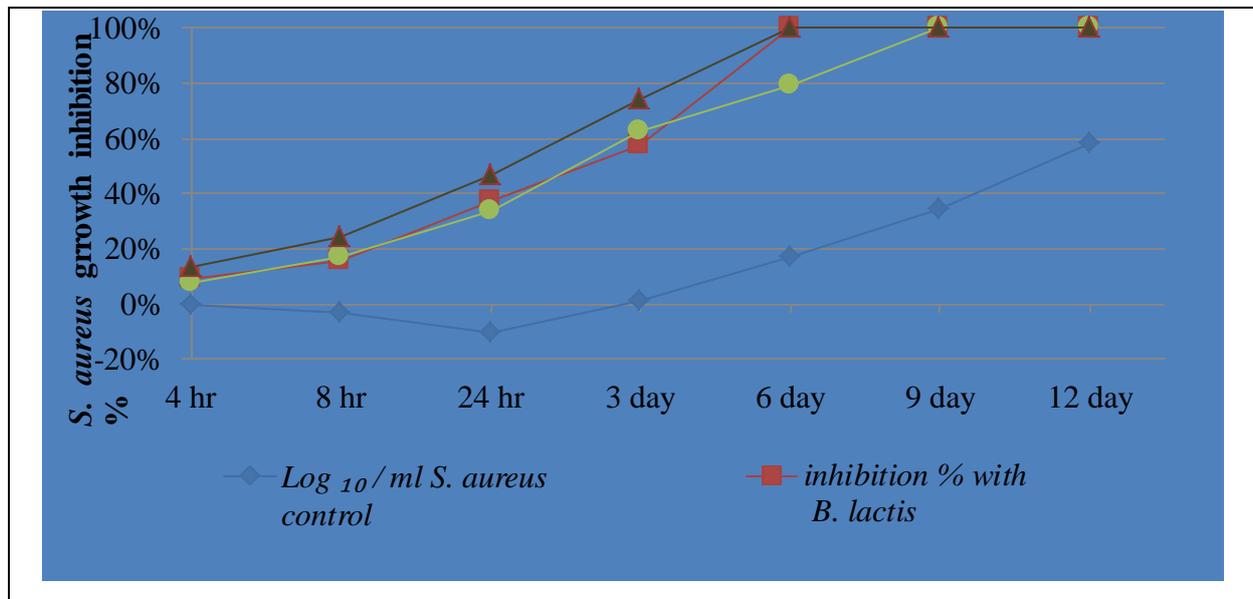


Figure 5 Growth inhibition % of *S. aureus* by *B. lactis*, yoghurt LAB starter culture and both *B. lactis* with yoghurt LAB starter culture compared to its growth alone in fresh milk during 12 days of storage at room temperature, after 4 hr incubation at 45° C

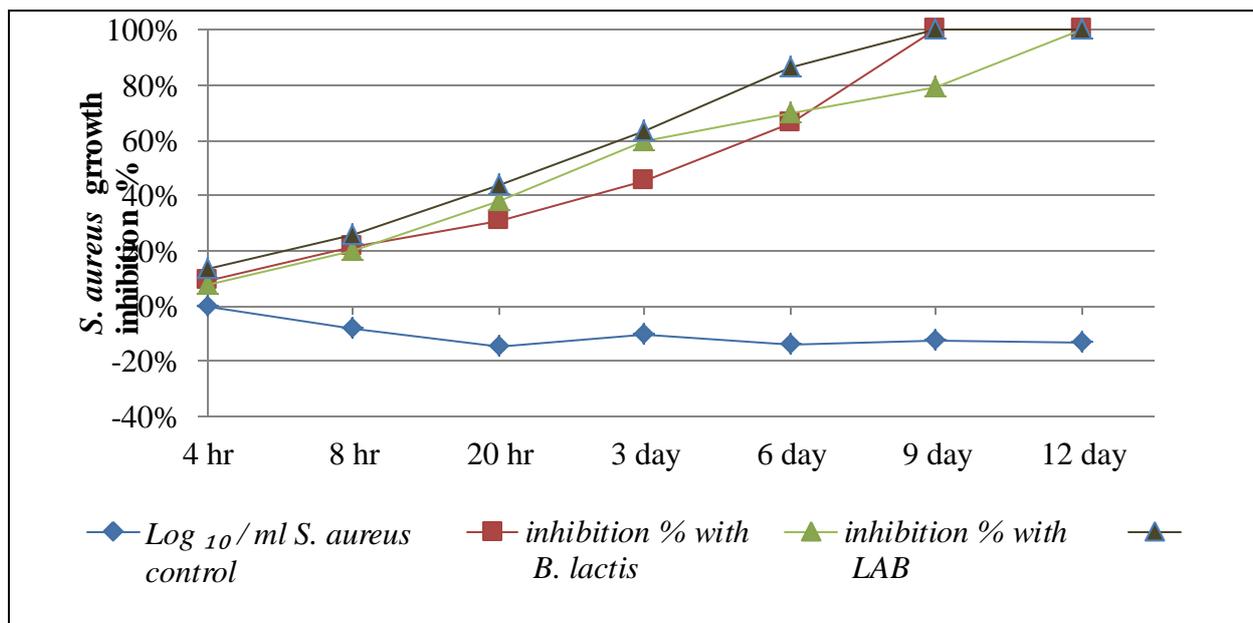


Figure 6 Growth inhibition % of *S. aureus* by *B. lactis*, yoghurt LAB starter culture and both *B. lactis* with yoghurt LAB starter culture compared to its growth alone in fresh milk during 12 days of storage at refrigerator temperature, after 4 hr incubation at 45° C

CONCLUSION

Yoghurt LAB starter culture significantly controlled and completely inhibited the growth of the pathogenic *S. aureus* (14) in milk during fermentation and storage at room and refrigeration temperatures. Similarly and more effective than LAB, *B. lactis* significantly controlled and completely inhibited the growth of *S. aureus* (14) in milk during fermentation and storage at room and refrigeration temperatures. The combination of LAB starter culture and *B. lactis* together (similar to *B. lactis*) significantly controlled and completely inhibited the growth of *S. aureus* (14) in milk during fermentation and storage. The pH values of milk were consistently decreased in all experiments during fermentation and storage periods.

REFERENCES

- ABEE, T. - CORCKLE, L. - HILL, C. 1995. Bacteriocins: modes of actions and potentials in food preservation and control of food poisoning. In *International Journal of Food Microbiology*, vol. 28, 1995, p. 169-185.
- BRIDSON, E.Y. 2006. The Oxoid Manual, (9th ed.), England, Oxoid limited.
- CAMASCHELLA, P. - MIGNOT, O. - PIROVANO, F. - SOZZI, T. 1998. Method for differentiated enumeration of mixed cultures of thermophilic lactic acid bacteria and *Bifidobacteria* by using only one culture medium. In *Lait*, vol. 78, 1998, p. 461-467.
- CHARLIER, C. - CRETENET, M. - EVEN, S. – LE LOIR, Y. 2009. Interaction between *Staphylococcus aureus* and lactic acid bacteria: An old story with new perspectives. In *International Journal of Food Microbiology*, vol. 131, 2009, p. 30-39.
- CLIVER, D. O. - RIEMANN, H. P. 2002. *Food Borne Diseases* (2nd ed.), California, Academic Press.
- CUNNIFF, P. 1995. *Official Methods of Analysis* (16th ed.), Virginia, AOAC International.
- INTERNATIONAL DAIRY FEDERATION (IDF) standard*, 117A:1988.
- JAY, J.M. 2000. *Modern Food Microbiology* (5th ed.), New York, International Thomson Publishing.
- KANDLER, O. - WEISS, N. 1986. *Bergey's Manual of Systematic Bacteriology*, Baltimore.
- LAHTINEN, S. J. - JALONEN, L. - OUWEHAND, A. C. - SALMINEN, S. J. 2007. Specific *Bifidobacterium* strains isolated from elderly subjects inhibit growth of *Staphylococcus aureus*. In *International Journal of Food Microbiology*, vol. 117, 2007, p. 125-128.

- LEE MARC, Y. - VALIK, L. - MEDVEDOVA, A. 2009. Modeling the effect of starter culture on the growth of *Staphylococcus aureus* in milk. In *International Journal of Food Microbiology*, vol. 129, 2009, p. 306-311.
- MARTINS, A. K. - MARTINS, F. S. - GOMES, D. A. - ELIAN, S. D. - VIEIRA, A. T. - TEIXEIRA, M. M. - CARA, D. C. - NARDI, R. M. - NICOLI, J. R. 2010. Evaluation of in vitro antagonism and in vivo immune modulation and protection against pathogenic experimental challenge of two probiotic strains of *Bifidobacterium animalis* var. *lactis*. In *Arch Microbiol*, vol. 192, 2010, p. 955-1003.
- RYBKA, S. - KAILASAPATHY, K. 1996. Media for the enumeration of yoghurt bacteria. In *International Dairy Journal*, vol. 6, 1996, p. 839-850.
- TABASCO, R. - PAARUP, T. - JANER, C. - PELAEZ, C. - REQUENA, T. 2007. Selective enumeration and identification of mixed cultures of *Streptococcus thermophilus*, *Lactobacillus delbrueckii* subsp. *bulgaricus*, *L. acidophilus*, *L. paracasei* subsp. *paracasei* and *Bifidobacterium lactis* in fermented milk. In *International Dairy Journal*, vol. 17, 2007, p. 1107-1114.
- TOPISIROVIC, L. - KOJIC, M. - FIRA, D. - GOLIC, N. - STRAHINIC, I. - LOZO, J. 2006. Potential of lactic acid bacteria isolated from specific natural niches in food and preservation. In *International Journal of Food Microbiology*, vol. 112, 2006, p. 230-235.