Bulgarian Journal of Agricultural Science, 15 (No 3) 2009, 196-203
Agricultural Academy

INFLUENCE OF LACTIC ACID BACTERIA ISOLATES ON *STAPHYLOCOCCUS AUREUS* GROWTH IN SKIMMED MILK

R. S. RADOVANOVIC* and V. KATIC
Department of Hygiene and Technology of Food of Animal Origin, Faculty of Veterinary Medicine, 11 000 Belgrade, Serbia

Abstract

RADOVANOVIC, R. S. and V. KATIC, 2009. Influence of lactic acid bacteria isolates on *Staphylococcus aureus* growth in skimmed milk. *Bulg. J. Agric. Sci.,* 15: 196-203

A total of 52 lactic acid bacteria (LAB) isolates was isolateded from acidcoagulating cheese samples one day old. The samples made from raw cows milk were collected from the houshold of an individual producer in Belgrade surroundings.

The isolates were identified as *Lactococcus* spp.(43.39%) and *Lactobacillus* spp. (54.71%) and were selected for additional identification. Out of 23 *Lactococcus* spp. isolates, by monitoring 24 different morphological and physiological characteristics, 18 (78.26%) isolates were identified as *Lactococcus lactis* subsp. *cremoris,* 2 (8.69%) isolates as *Lactococcus lactis* subsp.*lactis,* 2 (8.69%) isolates as *Lactococcus plantarum* and 1 (4.35) isolate as *Lactococcus lactis* subsp. *rafinolactis*. Out of 29 *Lactobcillus* spp. isolates from acidcoagulating cheese were observed for 19 different morphological and physiological properties; 9 (31.03%) isolates were identified as *Lactobacillus plantarum*, 4 (13.79%) isolates as *Lactobacillus brevis,* 3 (10.34%) isolates as *Lactobacillus buchneri,* 3 (10.34%) *Lactobacillus salivarius* subsp. *salivarius*, 3 (10.34%) isolates as *Lactobacillus acidophilus*, 2 (6.89%) isolates as *Lactobacillus paracasei* subsp. *tolerans,* 2 (6.89%) isolates as *Lactobacillus leichmanii,* 1 (3.45%) isolate as *Lactobacillus delbrueckii* subsp. *delbrueckii* and 1 (3.45%) isolate *Lactobacillus delbrueckii* subsp. *bulgaricus*. An inhibitory effect of LAB on *Staphylococcus aureus* growth was observed in skimmed milk. The resulting inhibition can be ascribed to the lowering of pH.

Key words: Lactic acid bacteria, *Lactococcus* spp., *Lactobacillus* spp., *Staphylococcus aureus*, cheese

Introduction

Lactic acid bacteria (LAB) are Gram-positive, usually non-motile, acid tolerant microorganisms. They have complex nutritional requirements and a fermentative metabolism. Phylogenetically the lactic acid bacteria belong to the clostridial branch of the Gram-positive bacteria. They are catalase negative, nonspore-forming, cocci, cocobacilli or rods that have less than 55 mol% G+C content in their DNA (Stiles and Holzapfel, 1997). Lactic acid bacteria are of importance in cheese making and ripening. These microorganisms can be devided into two main groups: the first group is one that can be added to cheese milk as a starter culture after being carefully selected by a starter manufacturer or a cheese making company,

* E-mail: mimica@vet.bg.ac.rs

and the second group represent nonstarter lactic acid bacteria (Peterson and Marshall, 1990; El Soda et al., 2000; Beresford et al., 2001; Somers et al., 2001). Both fermented dairy products and cheese play an important role in nutrition of people in Serbia. One of various cheese types produced in Serbia is white cheese (acidcoagulating and sweetcoagulating).This cheese can be consumed fresh or pickled. Fresh cheeses that are offered at Belgrade markets are usually one to seven days old. They are made of raw, or cooked cows milk by adding the rennet (or without rennet). Their producers do not add the starter culture into the milk, so the main microflora of cheeses are nonstarter lactic acid bacteria. Lactic acid bacteria occur in milk and are present in number which is capable to overgrow other microorganisms, so that is the basis of the traditional cheese manufacturing (Dimitrijevic-Brankovic et al., 2000). The literature provides evidence that green plant material is a natural source of lactococci (Salama et al., 1995).

The objective of this study was to isolate and identify lactic acid bacteria from this artisan, starter free cheese; i.e. to provide autochtonous strains as a positive dairy microflora, suitable to test their antagonistic activity against *Staphylococcus aureus* and that can be used to improve the hygienic quality of other raw milk cheeses .

Materials and Methods

Cheese samples: The cheese used in this study was made of raw cows milk consisting of a mixture of evening and morning milk. The samples were collected from the houshold of an individual producer in Belgrade surroundings (Sremcica). The cheese was collected one day after the curd had been formed. Coagulation was induced by adding of commercial rennet. Cheese portions (20g) were aseptically sampled and diluted in 180 ml sterile 2% sodiumcitrate solution. Aliquots of 1ml were taken, decimaly dilluted in physiological sterile saline, and plated on specific media required for the different microbiological species to be examined.

Isolation of LAB: M-17 agar (Merck) was used for isolation of *Lactococcus* spp.

MRS agar (Torlak) was used for isolation of *Lactobacillus* spp. The incubation for *Lactococcus* spp. was aerobically at 30°C for 24-48h, and for *Lactobacillus* spp. at 37°C for 48-72h. Characteristic colonies for lactococci that were Gram-positive cocci in chains, catalase negative, were inoculated into sterile skimmed milk and incubated at 30°C for 24h. Chracteristic colonies for lactobacilli that were Gram-positive rods in chains were inoculated into MRS broth incubated at 37°C for 48h. The isolated strains were kept in the freege at 4°C for futher studies; lactococci in sterile skimed milk and lactobacilli in MRS broth.

Identification of LAB was performed according to the criteria of Bergeyr's manual of determinitive bacteriology (Holt et al., 1994) and using the methods and criteria of Sharpe (Sharpe,1979), determining the macromorphological, phenotypic and biochemical characteristics.

Isolation of Staphylococcus aureus: Baird-Parker agar (Torlak) was used for isolation of *Staphylococcus aureus*.

pH in sterile reconstituted skimmed milk inoculated with *S. aureus* (S) and the screened strains of LAB were measured (pH-metar MA 5735-Iskra) after incubation at 30°C for 2, 4, 6, 8, 24 h.

Influence of LAB on Staphylococcus aureus in sterile skimmed milk was observed in mono and mix cultures. *Staphylococcus aureus* (S), isolated from acidcoagulating cheese, was chosen for examination in mono and mix cultures. LAB were se-

Table 1
Results of identification of *Lactococcus* spp. isolates

Isolate	n	%
Lactococcus lactis subsp.cremoris	18	78.26
Lactococcus lactis subsp.lactis	2	8.69
Lactococcus plantarum	2	8.69
Lactococcus raffinolactis	1	4.35
Total	23	100

lected on the basis of the pH decrease in the sterile reconstituted skimmed milk and their biochemical characteristics. The remaining strains were further identified and characterized by api 50 CH (Bio Merieux)

The size of the experimental inoculum was determined on the basis of their occurrence in the milk. In mono culture inoculum of 1ml overnight *Staphylococcus aureus* (S) broth culture (10^{-6}) was inoculated in 9ml sterile skimmed milk. The total number of *Staphylococcus aureus* (S) was determined after 2, 4, 6, 8 and 24h of incubation at 37°C and plating of 0.1ml on the BP agar surface from the decimal dilutions. The behaviour of LAB in monoculture was observed after inoculation of 1ml overnight broth culture(10^{-6}) in 9 ml sterile skimmed milk. The total number of LAB was determined after 2, 4, 6, 8 and 24 h of incubation at 30°C and inoculation decimal dilution into M-17 agar and MRS agar and incubation.

The study of the behaviour of *Staphylococcus aureus* (S), *Lactococcus lactis* subsp. *lactis* (30) spp. and *Lactobacillus plantarum* (39) in mix culture was carried out by inoculation of 1ml overnight

$$G = \frac{t}{\dfrac{\log b - \log B}{\log 2}} = \frac{t}{3.3\log\left(b/B\right)}$$

broth culture of all three microorganisms (1:1:1) in 9 ml sterile skimmed milk. The incubation was provided for 2, 4, 6, 8 and 24 h at 30°C, and the total count of *Staphylococcus aureus* (S) was determined on BP agar, *Lactococcus* spp. on M-17 agar and *Lactobacillus* spp. on MRS agar.

Generation time was determined for *Staphylococcus aureus* (S), *Lactococcuslactis* subsp. *lactis* (30) and *Lactobacillus plantarum* (39) in mono and mix cultures in sterile skimmed milk after 4, 6 and 8 h incubation at 30°C. The generation time was defined as a time needed for one cell to divide into two daughter cells and was counted by the following equation:

Where: G-one generation time; B-initial number of bacteria; b-number of bacteria at the end of given time; t- given time period.

Results

A total of 52 lactic acid bacteria (LAB) isolates was isolated from samples of acidcoagulating cheese made from raw milk, one day old. Of the isolates 23 (43.39%) belonged to *Lactococcus* species and 29 (54.71%) isolates to *Lactobacillus* species.

Among 23 lactococci isolates (Table 1) through the examination of of 24 differrent morphological and physiological characteristics, the predominant species was *Lactococcus lactis* subsp. *cremoris* (78.26%), then *Lactococcus lactis* subsp. *lactis* (8.69%), *Lactococcus plantarum* (8.69%) and *Lactococcus raffinolactis* (4.35%). Out of 29 *Lactobacillus* spp. isolates through the examination of 19 different morphological and physiological characteristics 9 (31.03%) isolates were identified as *Lactobacillus plantarum*, 4 (13.79%) isolates as *Lactobacillus brevis,* 3 (10.34%) isolates as *Lactobacillus buchneri,* 3 (10.34%) *Lactobacillus salivarius*

Table 2
Results of identification of *Lactobacillus* spp. isolates

Isolate	n	%
Lactobacillus plantarum	9	31.03
Lactobacillus brevis	4	13.79
Lactobacillus salivarius subsp.salivarius	3	10.34
Lactobacillus buchneri	3	10.34
Lactobacillus acidophilus	3	10.34
Lactobacillus paracasei subsp.tolerans	2	6.89
Lactobacillus leichmanii	2	6.89
Lactobacillus casei	1	3.45
Lactobacillus delbrueckii subsp. delbrueckii	1	3.45
Lactobacillus delbrueckii subsp.bulgaricus	1	3.45
Total	29	100

subsp. *salivarius*, 3 (10.34%) isolates as *Lactobacillus acidophilus*, 2 (6.89%) isolates as *Lactobacillus paracasei* subsp. *tolerans,* 2 (6.89%) isolates as *Lactobacillus leichmanii,* 1 (3.45%) isolate as *Lactobacillus delbrueckii* subsp. *delbrueckii* and 1 (3.45%) isolate *Lactobacillus delbrueckii* subsp. *bulgaricus* (Table 2).

The results of the study of behaviour of *S. aureus* (S), *Lactococcus lactis* subsp. *lactis* (30) and *Lactobacillus plantarum* (39) populations in mono culture are shown in Figure 1. The populations of *S. aureus* (S), *Lactococcus lactis* subsp. *lactis* (30) and *Lactobacillus plantarum* (39) in mono culture inoculated in skimmed milk multiplied during the time. The initial inoculum for *S. aureus* (S) was 1.47 log CFU/ml and after 4 h the number increased for 1 logarithmic value. After 6 h the number of *S. aureus* (S) was 3.50 log CFU/ml and after 8 h it increased 3.37 times from the initial inoculum. After 24 h incubation at 30°C the population of *S. aureus* (S) was present in 7.93 CFU/ml. The initial inoculum for *Lactococcus lactis* subsp. *lactis* (30) was 2.46 CFU/ml and in 2 h slightly increased. After 4 h the number of *Lactococcus lactis* subsp. *lactis* (30) decreased for 1.1 logarithmic value from the initial inoculum. After 6 and 8h the number of lactococci increased for aproximately 1 logarithmic value. After 24 h of incubation the number reached 8.54 log CFU/ml.

The population of *Lactobacillus plantarum* (39) in monoculture with the initial inoculum of 1.56 log CFU/ml multiplied during the time. After 4 h it decreased for 1.4 logarithmic value from the initial number. The tendency of an increase in the *Lactobacillus plantarum* (39) population continued and after 6 h the bacterium was present in the number of 4.13 log CFU/ml. After 8 h of incubation the number of lactobacilli decreased for 3.34 logarithmic value from the initial inoculum. After 24 h the number of *Lactobacillus plantarum* (39) increased to 7.39 log CFU/ml

The results cocerning the influence of LAB on *S. aureus* (S) after the inoculation of the mix culture in skimmed milk are shown in Figure 2. It is obvious that *S. aureus* (S) with the same inoculum of 1.47 log CFU/ml multiplied more slowly in the mix culture than in the monoculture. After 4h the population of *S. aureus* (S) decreased for 0.75 logarithmic value from the initial number. During the time the number of *S. aureus* (S) slightly decreased, after 6 h for 2.25 and after 8 h for 2.79 logarithmic value from the initial inoculum. After 24h the number of *S. aureus* (S) was 5.92 log CFU/ml.

The initial inoculum for *Lactococcus lactis* subsp. *lactis* (30) in the mix culture was 2.35 log CFU/ml and during the time it increased. After 2 h the number slightly increased to 2.82 log CFU/ml. After 4, 6 and 8 h the population of *Lactococcus lactis* subsp. *lactis* (30) increased for aproximately for 1 logarithmic value. After 24 h it reached 8.70 log CFU/ml.

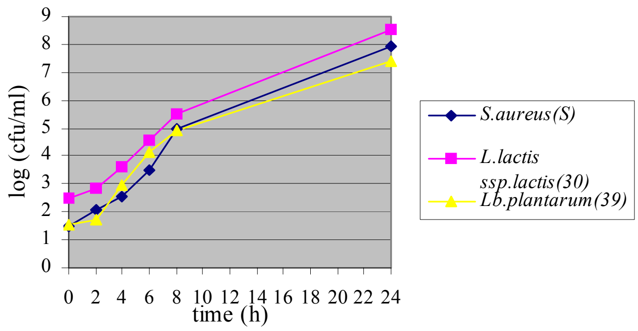

Fig. 1. Changes in population of *S. aureus* (S), *Lactococcus lactis* subsp. *lactis* (30) and *Lactobacillus plantarum* (39) in monoculture in sterile skimmed milk at 30°C

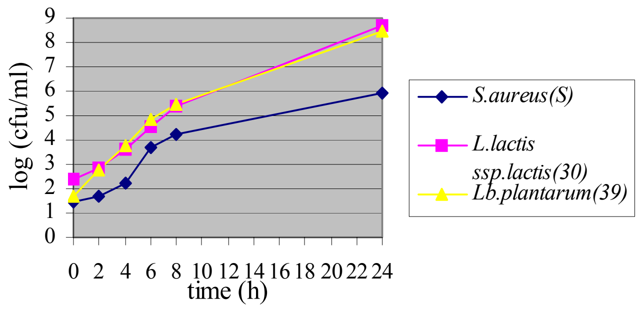

Fig. 2. Changes in population of *S. aureus* (S), *Lactococcus lactis* subsp. *lactis* (30) and *Lactobacillus plantarum* (39) in mix culture in sterile skimmed milk at 30°C

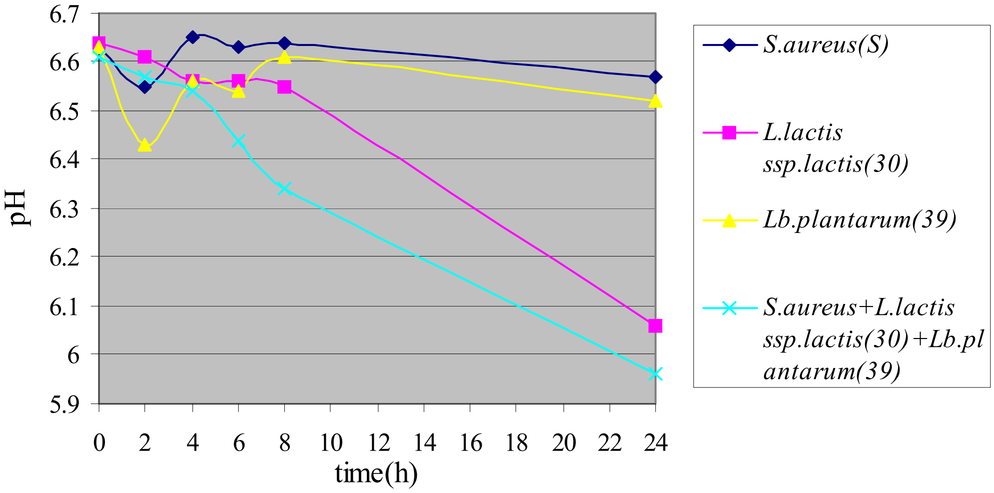

Fig. 3. Changes in pH values of sterile skimmed milk inoculated with mono and mix cultures of *S. aureus* (S), *Lactococcus lactis* subsp. *lactis* (30) and *Lactobacillus plantarum* (39)

The initial inoculum for *Lactobacillus plantarum* (39) in the mix culture was 1.66 log CFU/ml. After 2, 4, 6, 8 h the population decreased for 1 logarithmic value, After 24 h the incubation number of *Lactobacillus plantarum* (39) reached 8.44 log CFU/ml.

The results of changes in pH values of skimmed milk experimentaly inoculated with mono and mix cultures are shown in Figure 3.

S. aureus (S) in a mono culture did not significantly change the pH value of skimmed milk after 2, 4, 6, 8 h of incubation at 30°C. After 24 h incubation pH was 6.57. A slight decrease in pH value of skimmed milk inoculated with *Lactococcus lactis* subsp. *lactis* (30) monoculture was from 6.64 to 6.06. *Lactobacillus plantarum* (39) in a monoculture did not significantly change the pH of inoculated skimmed milk, and after 24h it reached 6.52. A decrease in pH of skimmed milk was in a mix culture from 6.61 to 5.96 after 24h of experimental inoculation.

The results of determination of generation time for *S. aureus* (S), *Lactococcus lactis* subsp. *lactis* (30) and *Lactobacillus plantarum* (39) in mono and mix cultures are shown in Table 3.

The longest generation time for *S.aureus* (S) was between 6 and 8 h of incubation at 30°C in mix cultures. Generation time for *Lactococcus lactis* subsp. *lactis* (30) in monoculture was longer after 6-8 h of incubation than between 4-6 h. The longest generation time for *Lactococcus lactis* subsp. *lactis* (30) in mix cultures was between 6-8 h. Generation time for *Lactobacillus plantarum* (39) in mono and mix cultures was longer between 6-8 h.

Discussion

The identified species and subspecies of lactic acid bacteria represent a typical population present in artisan cheeses. Simmilar results were obtained by Cogan et al. (1997) and Medina et al. (2001).The obtained results showed that among lactococci the predominant species was *Lactococcus lactis* subsp. *cremoris* and this correlates with the results by Poznanski et al. (2004). Our results differ from the results obtained by Poullet et al. (1993), Tornadijo et al. (1995), Arizcun et al. (1997) and Zaratea (1997), Zamfir et al. (2006), who isolated mostly *Lactococcus lactis* subsp. *lactis*. Salama et al. (1995) isolated *Lactococcus lactis* subsp. *cremoris* from raw milk and cottage cheese and Dimitrijevic-Brankovic et al. (2000) from Sjenicki cheese. The finding of one *L. raffinolactis* isolate in our results correlates with Scleifer et al. (1985) opinion.

In 29 *Lactobacillus* isolates the predominant species was *Lactobacillus plantarum*, and these results

Table 3
Generation time for *S. aureus* (S), *Lactococcus lactis* subsp. *lactis* (30) and *Lactobacillus plantarum* (39) in mono and mix cultures at 30°C

Species of microorganisms	Generation time, min			
	Mono culture		Mix culture	
	4-6 h	6-8 h	4-6 h	6-8 h
S. aureus (S)	36.81	24.68	24.10	66.79
L. lactis subsp. lactis (30)	36.80	39.24	37.51	43.83
Lb. plantarum (39)	30.62	47.03	33.70	53.93

Lopez and Mayo (1997), Dimitrijevic-Brankovic (2000), Medina et al. (2001), Aquilanti et al. (2006).

During cheese ripening possible pathogens are normally inhibited by LAB (Nunez et al., 1985). From one point of view LAB inhibited *S. aureus* and enterotoxin synthesis (Fang et al., 1996; Pinto et al., 1996; Gonzales-Fandos, 1997). Whereas, the other group of authors (Spillmann et al., 1978; Santos and Genigeorgis, 1981) did not find the inhibitory effect of LAB on *S. aureus.* Our results showed that LAB isolates exibited an inhibitory effect on *S. aureus* in experimentally inoculated skimmed milk. The inhibitory effect of LAB depends on lactose consumption with a consequent lactic acid production and pH reduction that creates a less favourable environment for pathogen growth (Ogava et al., 2001). Our results showed that the strongest inhibitory effect was probably due to lowering of pH. The reduction in the content of *S. aureus* also depend on bacteriocin production (Olasupo et al., 1999; Arques et al., 2005).

Conclusions

The isolated strains of lactic acid bacteria from acidcoagulating cheese, one day old, made of raw cows milk, represent typical microbiota for atisan cheeses. Among lactococcal species predominated *Lactococcus lactis* subsp. *cremoris* (78.26%) and among lactobacilli *Lactobacillus plantarum* (31.03%). The study of the effect of the isolates, chosen according to their acidification properties showed an inhibitory effect on *Staphylococcus aureus* in skimmed milk. The strongest inhibitory effects of *Lactococcus lactis* subsp. *lactis* (30) and *Lactobacillus plantarum* (39) on *Staphylococcus aureus* were in the mix culture after the incubation for 24 h at 30°C. This explains the longest generation time for *S. aureus* in the mix culture in skimmed milk between 6 and 8 h of incubation at 30°C. The inhibitory effect of lactic acid bacteria isolates was probably due to the lowering of the pH of inoculated skimmed milk.

References

Aquilanti, L., L. Dell Aquila, E. Yannini, A. Zocchetti and F. Clementi, 2006. Resident lactic acid bacteria in raw milk Canestrato Pugliese cheese. *Lett Appl Microbiology*, **43** (2): 161-167.

Arques, I. L., E. Rodriguez, G. Pilar, M. Margarita, B. Guamis and M. Nunez, 2005. Inactivation of *Staphylococcus aureus* in raw milk cheese by combinations of high pressure treatments and bacteriocin producing lactic acid bacteria. *J. Appl. Micr.*, **98** (2): 254-260.

Arizcun, C., Y. Barcina and P. Torre, 1997. Identification of lactic acid bacteria isolated from Roncal and Idiazabal cheeses. *La Lait*, **77**: 729-736.

Bergeys Manual of Systematic Bacteriology, 1986. *Wiliams and Wilkins*, Baltimore/ London, **2:** 1013-1019, 1209-1234, 1065-1066.

Beresford, T. P., N. A. Fitzsimons, N. L. Brennan and T. M. Cogan, 2001. Recent advances in cheese microbiology. *Int. Dairy J.*, **11**: 259-274.

Cogan, T. M., M. Barbosa, E. Beuvier, B. Bianchi-Salvadoriset, P. S. Cocconcelli, I. Fernandes,

J. Gomez, R. Gomez, G. Kalantzoupoulos, A. Ledde, M. Medina, M. C. Rea, and E. Rodriguez, 1997. Characterization of the lactic acid bacteria in artisan dairy products. *J. Dairy Research*, **64**: 409-421.

Dimitrijevic-Brankovic,S.,J. Baras and A. Banina, 2000. Identification and Characterisation of Natural Lactic Microflora of Home Made Cheese from Sjenica. *J. Sci. Agricul. Res.*, **1-2**: 329-341.

El Soda, M., A. S. Medkor and P. S. Tong, 2000. Marschall Rhodia International Dairy Science Award Lecture. Adjuncnt Cultures: Recent Developments and Potential Significance to the Cheese industry. *J. Dairy Science,* **83:** 609-619.

Fang, W., M. Shi, L. Huang, J. Chen and Y. Wang, 1996. Antagonism of lactic acid bacteria towards *Staphylococcus aureus* and *Escherichia coli* on agar plates and in milk. *Vet. Res.*, **27 (1)**: 3-12.

Gonzales-Fandos, M. E., M. Sierra, M. L. Garcia-Lopez, M.F. Fernandez-Alvarez, M. Prieto and A. Otero, 1997. Effect of lactic acid bacteria on growth of *Staphylococcus aureus* and enterotoxins (A-D) and thermonuclease production in broth. *Archiv fur Lebensmittelhygiene*, **48**: 38-41.

Holt, et al., 1994. In: Bergeys Manual of Determinative Bacteriology, Ninth Edition, *Wiliams and Wilkins*, pp.528-529, 532, 536, 540, 544,566.

Lopez, S. and B. Mayo, 1997. Identification and characterization of homofermentative mesophilic *Lactobacillus* strains isolated from artisan starter-free cheeses. *Lett. in Appl. Micr.*, **25:** 233-238.

Medina, R., M. Katz, S. Gonzales and G. Oliver, 2001. Characterization of the lactic acid bacteria in ewes milk and cheese from northwest Argentina. *J. of Food Protection*, **64** (4): 559-563.

Nunez, M., P. Gaya and M. Medina, 1985. Influence of manufacturing and ripening conditions *on the survival of Enterobacteriaceae* in Manchego cheese. *J. of Dairy Science,* **68**: 794-800.

Ogawa, M., K. Shimizu, K. Nomoto, R. Tanaka, T. Hamabata, S. Yamasaki, T. Takeda and Y. Takeda, 2001. Inhibition of *in vitro* growth of Shiga toxin-producing *Escherichia coli* O157:H7 by probiotic *Lactobacillus* strains due to production of lactic acid. *Int.J. Food Micr.* **68**: 135-14.

Olasupo, N. A., U. Schillinger, A. Narbad, H. Dodd and W. H. Holzapfel, 1999. Occurrence of nisin Z production in *Lactococcus lactis* BFE 1500 isolated from wara, a traditional Nigerian cheese product. *Int. J. Food Micr.*, **53**: 141-152.

Peterson, S. D., R. T. Marshall and H. Heyman, 1990. Peptidase profiling of lactobacilli associated with Cheddar cheese and its application to identification and selection of strains of cheeseripening studies. *J. of D. Science,* **73**: 1454-1464.

Pinto, M. F., E. H. G. Ponsano and R. J. H. Castro-Gomez, 1996. Antibiosis associated with growth of *Lactobacillus axcidophilus.* 1. Strain selection and study of analternative culture medium based on whey. *Arquivos de Biologia e Tecnologia*, **39** (**2**): 247-257.

Poulett, B., M. Huertas, A. Sanchey, P. Caceres and G. Larriba, 1993. Main lactic acid bacteria isolated during ripening of Casar de Caceres cheese. *J. Dairy Research*, **60**: 123-127.

Santos, E. C. dos, C. Gengigeorgis and T. B. Ferver, 1981. Prevalence of *Staphylococcus aureus* in raw and pasteurized milk used for commercial manufacturing of Brazilian Minas cheese. *J.Food Protection*, **44** (3): 172-176.

Salama, M. S., T. Mustafija-Jeknic, W. E. Sandine and S. J. Giovannoni, 1995. An ecological study of lactic acid bacteria: Isolation of new strains of *Lactococcus* including *Lactococcus lactis* subspecies *cremoris. J. Dairy Science*, **78**: 1004-1017.

Sharpe, M. E., 1979. Identification of lactic acid bacteria in Identification Methods for Microbiologist. Soc. Appl. Bacteriol. Technical series 14, 2nd edn (FA Skinnner, DW Levelock Eds) *Academic Press,* London, pp. 246-255.

Schleifer, H. K., R. Kilpper-Balz, D. M. Collins and W. Fisher, 1985. Transfer of *Streptococcus lactis* and Related Streptpcocci to the Genus *Lactococcus* gen. nov. *System Appl. Microbiology*, **6**: 183-195.

Somers, E. B., M. E. Johnson and A. C. L. Wong, 2001. Biofilm formation and contamination of cheese by nonstarter lactic acid bacteria in dairy environment. *J. Dairy Science*, **84**: 1926-1936.

Spillmann, H., Z. Puhan and M. Benhagyi, 1978. Antimicrobille Aktivitat thermophiler Lactobayillen. *Milchwissenschaft*, **33** (3): 148-153.

Stiles, M. E. and W. H. Holzapfel, 1997. Lactic acid bacteria of foods and their current taxonomy. *Int. J. Food Microbiology*, **36:** 1-29.

Tornadijo, M. E., J. M. Fresno, J. Carballo and R. M. Sermiento, 1996. Population levels, species and characteristics of micrococaceae during manufacturing and ripening of Armada-Sobado hard goats milk cheese. *J. Food Protection*, **59** (11): 1200-1207.

Zaratea, V., F. Belda, C. Perez and E. Cardell, 1997. Changes in Microbial-Flora of Tenerife Goats Milk Cheese during Ripening. *Int. Dairy . J.*, **7** (10): 35-87.

Zamfir, M., M. Vancanneyt, L. Makras, F. Vaningelgem, K. Lefebvre, B. Pot, J. Swings and L. De Vuyst, 2006. Biodiversity of lactic acid bacteria in Romanian daiy products. *Syst.. Appl. Microbiology*, **29** (6): 487-495.

Received January, 23, 2009; accepted for printing April, 2, 2009.