IJFANS
International Journal of Food And Nutritional Sciences

Volume 2, Issue-1, Jan-Mar-2013 www.ijfans.com ISSN: 2320-7876

INTERNATIONAL JOURNAL OF FOOD AND NUTRITIONAL SCIENCES

Official Journal of IIFANS

INTERNATIONAL JOURNAL OF FOOD AND NUTRITIONAL SCIENCES

ISSN 2320 –7876 www.ijfans.com
Vol.2, Iss.1, Jan-Mar 2013

Research Paper *Open Access*

EFFECT OF BACTERIOCIN EXTRACTED FROM *ENTEROCOCCUS FAECIUM BS* 13 ON SHELF LIFE OF PANEER AND KHOYA

Vandana Bali, Parmjit S. Panesar[1] and Manab B. Bera
Food Biotechnology Research Laboratory, Department of Food Engineering and Technology, Sant Longowal Institute of Engineering and Technology, Longowal, India.

[1]Corresponding author: pspanesarrr@yahoo.com

ABSTRACT

Bacteriocins are ribosomally synthesized natural metabolites, which act against food spoilage and pathogenic bacteria, thereby, extending the shelf life of food products. Bacteriocins from lactic acid bacteria are of prime focus nowadays due to their GRAS status. Keeping this in view, bacteriocin produced by *Enterococcus faecium* BS 13 (isolated from fermented berseem) was extracted and incorporated in samples of paneer and khoya to evaluate its biopreservative potential. Microbiological analysis was made after regular time intervals for a period of 30 days. Supplementation of bacteriocin BS 13 resulted in the extension of shelf life of paneer and khoya under refrigeration conditions as compared to control. The effect of addition of sorbic acid along with bacteriocin to the samples was also observed, however, the decrease in the bacterial load was comparable to bacteriocin alone. Moreover, the overall acceptance in terms of various characteristics of odour, colour and sliminess of paneer and khoya sample was more in bacteriocin and sorbic acid supplemented samples as compared to bacteriocin supplemented samples. The sensory analysis by the trained panelists considered the bacteriocin supplemented product to be more acceptable as compared to the control.

Keywords: *Enterococcus faecium*, bacteriocin, shelf-life extension, paneer, khoya.

INTRODUCTION

There has been increased demand of consumers, throughout the world, for healthier and ready-to-eat food products free from chemical preservatives due to their undesirable side-effects (Oguntoyinbo et al., 2007). Bacteriocins, known as potential biopreservatives, have become the focus area of academicians, researchers, and food industries. These are ribosomal-synthesized antimicrobial compounds which are proteinaceous in nature and act mostly against closely related species. These are being used as a tool to prevent and control the growth of food spoilage and pathogenic bacteria, thereby, extending the shelf life of food products and making them consumer acceptable (Deegan et al., 2006). Bacteriocins from Lactic acid bacteria (LAB) have gained special interest due to its "Generally Regarded As Safe" (GRAS) status. Bacteriocin producing LAB strains like *Lactococcus, Lactobacillus, Pediococcus, Enterococcus,* etc. can be used as alternative for chemical preservatives and starter cultures for food fermentation of milk/dairy, vegetable and meat products etc. (Nettles and Barefoot, 1993). *Enterococcus faecium* (producing enterocins) has been used as starter culture for various meat and dairy fermented products. They bear proteolytic and lipolytic activity, further contributing to the organoleptic properties in ripening of Mozzarella, Cheddar, Tallegia cheese (Giraffa et al., 1995; Tzanetakis and Litopoulou-Tzanetaki, 1992), sausages (Veljovic et al., 2009) and black olives (Franz et al., 1996).

India has variety of traditional foods and various fermented products like milk products, cereals, vegetables, fruits are some of the examples (Jeyaram et al., 2009; Thapa et al., 2004). India is the world's largest milk producer and consumer with market expected to surpass US $ 163 billion by 2017 (Indian Dairy Market Report & Forecast, 2012). Dairying has been a source of employment especially among the rain-fed and drought-prone regions of rural population. Dairy products like paneer, khoya, curd etc. (local Indian milk products) constitute low cost and nutritious food for a large segment of vegetarian Indian population. These products are the rich source of animal protein and calcium for this group population. Most of the dairy products have short shelf life and has been problematic for producer, processor, retailers and consumers. To overcome this problem, various bacteriocins have been isolated

purified and characterized from different LAB strains but their application in real system (food product) is the prime interest of the industry for its commercialization. Thus, the current study is focused on the evaluation of shelf life extension of ancient Indian dairy products i.e. paneer and khoya using bacteriocin obtained from *Enterococcus faecium* BS 13.

MATERIAL AND METHODS

All the experiments were performed in triplicate and their mean values have been reported.

MICROORGANISMS

Enterococcus faecium BS13 was isolated in the Biotechnology Research Laboratory and was maintained on MRS medium (Bali et al., 2011; 2012). The inoculum was prepared by incubating the flasks containing MRS in a rotary shaker at 30^{o}C overnight at 100 rpm.

BACTERIOCIN PRODUCTION AND EXTRACTION

Bacteriocin production was carried out in 250 ml Erlenmeyer flasks each containing 100 ml medium (malt extract (1%); beef extract (3.5%); $CaCl_2$ (0.75%) and 3-4 drops polysorbate 80) with 2% inoculum at 37 ^{o}C, pH 6.0 and 100 rpm for 18 hrs. For extraction of bacteriocin, the fermentation broth was centrifuged at 10,000 rpm for 10 min at 4^{o}C. Supernatant was filtered through pre-sterilized 0.22 µm filters (HiMedia, Mumbai) and pH was neutralized using 0.1N NaOH (Biswas et al., 1991). This crude bacteriocin was further used for estimating the extension of shelf life of food products.

PREPARATION OF SAMPLES

Freshly prepared paneer and khoya samples were procured from local market of Barnala (Punjab, India). The pieces (20 g) of 3cm x 3 cm with 0.5 mm thickness of both the samples were cut and dipped in crude bacteriocin alone and mixture of crude bacteriocin and 0.1% sorbic acid for 2 hrs. These were then wrapped in sterilized aluminum foil and stored under refrigerated conditions (4 °C) for 30 days.

MICROBIOLOGICAL ANALYSIS

The paneer and khoya samples were taken out at different time intervals for a period of 30 days (Table 1-2). For plate count of total aerobic bacteria, serial dilutions were prepared and plated on standard plate count agar. The plates were incubated at 37°C for 48 hrs and colonies were counted with the help of Darkfield Quebec Colony counter.

SENSORY EVALUATION

Sensory evaluation of samples was based on colour, odour, sliminess/spoilage, appearance and overall acceptance for their different quality attributes was rated on a 9-point hedonic scale by 6 trained panelists (Intarapichet and Gosaarak, 2008).

RESULTS AND DISCUSSION

MICROBIOLOGICAL ANALYSIS OF PANEER

Bacteriocins action as biopreservative helps in increasing the shelf life of the food products by providing safety from food pathogenic and spoilage microorganisms (O'Sullivan et al., 2002; Settanni and Corsetti, 2008; Settanni and Moschetti, 2010). The effectiveness of bacteriocin isolated from *E. faecium* BS 13 strain to act as biopreservative and its role in increasing shelf life of paneer and khoya was checked in presence of bacteriocin as compared to control and addition of chemical preservative (sorbic acid). In case of control sample, a slight increase in microbial count was observed up to 5 days followed by sharp increase in the count after 10 days of storage period, which could be due to the initiation of spoilage of paneer at 4^{o}C (Table 1). In case of bacteriocin supplemented paneer samples, microbial cell load was found to be 14×10^6 cfu ml^{-1} after treatment with bacteriocin which was less by factor of 10^4 as compared to the control samples (untreated). This could be due to bacteriocidal effect of bacteriocin on the microorganisms initially present in the sample (Samelis et al., 2003). After 10 days of storage, the microbial load was observed to be 56×10^{10} cfu ml^{-1}. The results depicted that the microbial load of bacteriocin treated samples after 10 days were comparable to initial control sample (zero hrs). The large increase in number of *L. monocytogenes* was observed in nisin free cheese sample as reported in earlier studies too (Govaris et al., 2001). Even after 15 days of storage, only 40×10^{11} microbes were observed as compared to 190×10^{15} in control. It has been previously reported that bacteriocin at concentration of 320 IU ml^{-1} and 160 IU ml^{-1} in pasteurized milk showed lowest total bacterial count and increased the shelf life upto 12 days of refrigerated storage (Ibrahim and Elbarbary, 2012). Lower concentration (40Uml^{-1}) is ineffective in preventing the spoilage of milk after 6 days of storage. Similar studies on decreased viable cell count in milk and soft-cheese had been reported by other authors (Bizani et al., 2008; Sable et al., 2000).

Different types of organic acids (sorbic acid, acetic acid, and lactic acid or potassium benzoate) have been used as antimicrobial agents (Bjorkroth, 2005; Georgnaras et al., 2006). Moreover, the bacteriocin in combination with different physical and chemical agents has also been used to increase the shelf life and safety of different food items (Alpas and Bozoglu, 2000; Munoza *et al.*, 2007). The experimentation on the combined effect of bacteriocin and sorbic acid on the paneer samples indicated a slight decrease in the microbial load as compared to bacteriocin supplemented samples due to antifungal nature of sorbic acid. The studies on the ripened Greek Graviera cheese using enterocin producing *E. faecium* also indicated decreased level of *Listeria monocytogenes* (Giannou et al., 2009). Samelis et al. (2003) also reported bactericidal effect of nisin treatment at day zero on *Listeria monocytogenes* in Anthotyros, a traditional Greek whey cheese stored at 4^{o}C. Addition of nisin (500 IU gm^{-1}) to whey is more effective in

decreasing pathogen level as compared to cheese and helped in increasing the preservation time up to 30-45 days.

Table 1: Effect of bacteriocin BS13 on microbial count in paneer during storage at 4°C

Day	Paneer	Paneer + Bacteriocin	Paneer + Bacteriocin + Sorbic acid
0	47×10^{10}	14×10^{6}	13×10^{6}
1	170×10^{10}	25×10^{6}	22×10^{6}
2	178×10^{10}	27×10^{6}	25×10^{6}
3	121×10^{11}	38×10^{7}	36×10^{7}
5	170×10^{11}	47×10^{8}	42×10^{8}
10	41×10^{12}	56×10^{10}	51×10^{10}
15	19×10^{16}	40×10^{11}	39×10^{11}
20	43×10^{16}	133×10^{13}	126×10^{13}
25	46×10^{16}	194×10^{14}	185×10^{14}
30	200×10^{18}	41×10^{15}	39×10^{15}

Table 2: Effect of bacteriocin BS13 on microbial count in Khoya during storage at 4°C

Day	Control	Khoya + Bacteriocin	Khoya + Bacteriocin + Sorbic acid
0	20×10^{2}	224	190
1	154×10^{2}	3×10^{2}	2×10^{2}
2	38×10^{3}	5×10^{2}	5×10^{2}
3	36×10^{4}	10×10^{2}	9×10^{2}
5	48×10^{4}	15×10^{2}	14×10^{2}
10	84×10^{4}	28×10^{2}	25×10^{2}
15	60×10^{5}	43×10^{3}	38×10^{3}
20	110×10^{7}	36×10^{5}	35×10^{5}
25	37×10^{8}	45×10^{6}	42×10^{6}
30	300×10^{9}	64×10^{7}	63×10^{7}

Wide variation in sensitivity of different pathogens especially *L. monocytogenes* to nisin was due to their characteristics, presence to nisin resistant mutants, different growth behaviors and other environmental conditions (De Martinis et al., 1997; Lioliou et al., 2001; Ming and Daeschel,1993; Ukuku and Shelef, 1997).

MICROBIOLOGICAL ANALYSIS OF KHOYA

The effect of bacteriocin and combined effect of bacteriocin and sorbic acid on shelf life of khoya is presented in Table 2. The total microbial count in khoya was 20×10^{2} as compared to 47×10^{10} in paneer at day zero. In the present study, initial lower microbial load in khoya as compared to paneer might be due to the heat treatment. Similarly previous studies indicate that the treatment of heat during the meatball preparation inactivates vegetative cells, which was indicated by zero microbial count in control and treated samples at day zero (Intarapichet and Gosaarak, 2008). The data also shows the bactericidal effect of bacteriocin as drastic decrease in viable cell count in initial sample (day zero) on addition of bacteriocin alone or combined mixture of bacteriocin and sorbic acid. In initial sample (day zero), approximately 90% decrease in viable cell count was observed after bacteriocin treatment as compared to control. After 20 days of storage, bacteriocin treated sample showed 36×10^{5} viable cell count as compared to 110×10^{7} in the control. The shelf life of sample was increased upto 20 days during the storage. Enterocin CCM 4231 at concentration of 3200 AU ml^{-1} has also shown bactericidal effect against *L. monocytogenes* and bacteriostatic to *Staphylococcus aureus* in soy milk (Laukova and Czikkova, 1999). Crude bacteriocin from *Lactococcus lactis* TISTR 1401 prevented the growth of total aerobic bacteria up to day 6 in treated meatball batter as compared to control (Intarapichet and Gosaarak, 2008).

Comparable effect on addition of sorbic acid with bacteriocin was observed during the storage of khoya sample as compared to bacteriocin alone as observed in Table 2. After 20 days of storage, viable bacterial count of 36×10^{5} and 35×10^{5} was there in bacteriocin and bacteriocin + sorbic acid treated samples respectively. It has also been reported that sorbic acid and sorbates had antifungal effect; however, these were not much effective against bacterial load (Han, 2005). While evaluating the effect of nisin, organic acids, salts, alone and in combination with each other on preservation of vacuum packaged sliced pork bologna at 4°C, nisin in combination with sodium diacetate (3g/100ml) has been found more effective in controlling *Listeria monocytogenes* (Samelis et al., 2005).

Characteristics of product: Various characteristics of paneer and khoya during storage in presence of bacteriocin and mixture of bacteriocin and sorbic acid are represented in Table 3 (a & b). The colour and odour of the paneer and khoya was maintained well upto 15 and 20 days, respectively as compared to control, however sliminess in bacteriocin treated sample was observed as compared to bacteriocin and sorbic acid supplemented samples. This indicated the additional inhibitory action of sorbic acid in preservation. As shown in Table 1, the level of microbial load is quite low in bacteriocin treated samples at day 15, but decreased acceptance level led to conclusion that microorganism other than bacteria play role in spoilage of food items. Similar effect on characteristics like color and sensory behavior was observed in study of bacteriocin in aerobically packed pork meatballs (Intarapichet and Gosaarak et al., 2008).

CONCLUSIONS

Bacteriocin production have become most focused and interesting area of research from last few decades in the area of food research for extension of shelf life of food products. Surface application of crude bacteriocin produced by *Enterococcus faecium* BS 13 extended the shelf life of paneer and khoya samples up to 15 and 20 days, respectively under refrigerated conditions.

Table 3a: Effect of bacteriocin BS13 and sorbic acid on the characteristics of paneer

Days	Treatment	Parameters Rating			
		Colour	Odour	Sliminess	Overall acceptance
0	**C**	9	9	0	9
	B	9	9	0	9
	BSA	9	9	0	9
1	**C**	9	9	0	9
	B	9	9	0	9
	BSA	9	9	0	9
2	**C**	9	9	0	9
	B	9	9	0	9
	BSA	9	9	0	9
5	**C**	7	7	2	7
	B	9	9	0	9
	BSA	9	9	0	9
10	**C**	5	5	5	3
	B	9	9	0	9
	BSA	9	9	0	9
15	**C**	1	1	9	0
	B	8	7	2	7
	BSA	8	7	1	8
20	**C**	0	0	9	0
	B	5	3	8	3
	BSA	6	4	6	4
25	**C**	0	0	9	0
	B	5	2	9	0
	BSA	5	3	9	0
30	**C**	0	0	9	0
	B	0	0	9	0
	BSA	0	0	9	0

C: Control; B: Bacteriocin supplemented; BSA: Bacteriocin + Sorbic acid supplemented

Table 3b: Effect of bacteriocin BS13 and sorbic acid on the characteristics of khoya

		Parameters Rating			
Days	**Treatment**	**Colour**	**Odour**	**Sliminess**	**Overall acceptance**
0	**C**	9	9	0	9
	B	9	9	0	9
	BSA	9	9	0	9
1	**C**	9	9	0	9
	B	9	9	0	9
	BSA	9	9	0	9
2	**C**	9	9	0	9
	B	9	9	0	9

	BSA	9	9	0	9
5	**C**	9	9	0	9
	B	9	9	0	9
	BSA	9	9	0	9
10	**C**	8	8	1	8
	B	9	9	0	9
	BSA	9	9	0	9
15	**C**	4	3	4	0
	B	9	9	0	9
	BSA	9	9	0	9
20	**C**	0	0	9	0
	B	7	7	7	7
	BSA	8	7	7	8
25	**C**	0	0	9	0
	B	6	4	6	2
	BSA	6	5	7	3
30	**C**	0	0	9	0
	B	0	0	9	0
	BSA	0	0	9	0

C: Control; B: Bacteriocin supplemented; BSA: Bacteriocin + Sorbic acid supplemented

Moreover, shelf life of paneer and khoya samples inoculated with bacteriocin and sorbic acid (0.1%, w/v) was also increased up to 15 and 20 days respectively as compared to control. However, sensory analysis revealed that bacteriocin and sorbic acid treated product was better than bacteriocin alone in terms of colour, odour, less contamination and acceptability. Thus, this bacteriocin has good potential for its use as biopreservative in food products.

ACKNOWLEDGEMENT

Vandana Bali is thankful to DST, New Delhi for providing INSPIRE Fellowship for carrying out present work.

REFERENCES

- Alpas, H. and F. Bozoglu, 2000. The Combined Effect of High Hydrostatic Pressure, Heat and Bacteriocins on Inactivation of Foodborne Pathogens in Milk and Orange Juice. World Journal of Microbiology and Biotechnology, 16: 387-392.
- Bali, V., P.S. Panesar and M.B. Bera, 2011. Isolation, Screening and Evaluation of Antimicrobial Activity of Potential Bacteriocin Producing Lactic Acid Bacteria Isolate. Microbiology Journal, 1(3): 113-119.
- Bali, V., P.S. Panesar and M.B. Bera, 2012. Physiological, Biochemical and Molecular Characterization of Potential Bacteriocin Producer Strain Isolated from Fermented Berseem. Acta Alimentaria. (Accepted Manuscript).
- Biswas, S.R., P. Ray, M.C. Johnson and B. Ray, 1991. Influence of Growth Conditions on the Production of a Bacteriocin AcH by *Pediococcus acidilactici* H. Applied and Environmental Microbiology, 57: 1265-1267.
- Bizani, D., J.A. Morrissy, A.P. Dominguez and A. Brandelli, 2008. Inhibition of *Listeria monocytogenes* in Dairy Products Using the Bacteriocin Like Peptide Cerein 8A. International Journal of Food Microbiology, 31: 229-233.
- Bjorkroth, J., 2005. Microbiological Ecology of Marinated Meat Products. Meat Science, 70: 477-480.
- Deegan, L.H., P.D. Cotter, C. Hill and P. Ross, 2006. Bacteriocins: Biological Tools for Biopreservation and Shelf-life Extension. International Dairy Journal, 16: 1058-1071.
- De Martinis, E.C.P., A.D. Cranda, A.S. Mazzotta and T.J. Montville, 1997. Influence of pH, Salt and Temperature on Nisin Resistance of *Listeria monocytogenes*. Journal of Food Protection, 60: 420-423.
- Franz, C.M.A.P., U. Schillinger and W.H. Holzapfel, 1996. Production and Characterisation of Enterocin 900, a Bacteriocin Produced by *Enterococcus faecium* BFE 900 from Black Olives. International Journal of Food Microbiology, 29: 255-270.
- Geornaras, I., P.N. Skandamis, K.E. Belk, J.A. Scanga, P.A. Kendall, G.C. Smith and
- J.N. Sofos, 2006. Post-processing Application of Chemical Solutions for Control of *Listeria monocytogenes*, Cultured Under Different Conditions, on Commercial Smoked Sausage Formulated With and

Without Potassium Lactate-Sodium Diacetate. Food Microbiology, 23: 762-771.

- Giannou, E., A. Kakur, B.B. Matijasic, I. Rogelj and J. Samelin, 2009. Fate of *Listeria monocytogenes* on Fully Ripened Greek Graviera Cheese Stored at 4, 12, or 25 degrees C in Air or Vacuum Packages: *in situ* PCR Detection of a Cocktail of Bacteriocins Potentially Contributing to Pathogen Inhibition. Journal of Food Protection, 72: 531-538.
- Giraffa, G., N. Picchioni, E. Neviani and D. Carminati, 1995. Production and Stability of an *Enterococcus faecium* Bacteriocin During Taleggio Cheese Making and Ripening. Food Microbiology, 12: 301-307.
- Govaris, A., P. Koidis and K. Papatheodorou, 2001. The Fate of *Escherichia coli* O157:H7 in Myzithra, Anthotyros, and Manouri Whey Cheeses during Storage at 2 and 12 ^{o}C. Food Microbiology, 18: 565-570.
- Han, J.H., 2005. Antimicrobial packaging systems. In Innovations in food packaging, Eds., Jung H. Han, Amsterdam: Elsevier Academic Press, pp. 81-107.
- Ibrahim, E.M.A. and H.A. Elbarbary, 2012. Effect of Bacteriocin Extracted from *Lactobacillus acidophilus* on the Shelf-life of Pasteurized Milk. Journal of American Science, 8(2): 620-626.
- Indian Dairy Market Report & Forecast, 2012. International Market Analysis Research & Consulting (http://www.imarcgroup.com/indian-dairy-market-report-forecasts)
- Intarapichet, K. and S. Gosaarak, 2008. The Use of Crude Bacteriocins From *Lactococcus lactis* TISTR 1401 as Biopreservative to Extend Shelf Life of Aerobically Packed Pork Meatballs. www.icomst.helsinki.fi/ICoMST2008/.../2A/2A.5.Intarapichet.pdf
- Jeyaram, K., T.A. Singh, W. Romi, A.R. Devi and W.M. Singh, 2009. Traditional Fermented Foods of Manipur. Indian Journal of Traditional Knowledge, 8: 115-121.
- Laukova, A. and S. Czikkova, 1999. The Use of Enterocin CCM 4231 in Soy Milk to Control the Growth of *Listeria monocytogenes* and *Staphylococcus aureus*. Journal of Applied Microbiology, 87: 182-186.
- Lioliou, K., E. Litopoulou- Tzanetaki, N. Tzanetakis and RK Robinson, 2001. Changes in the Microflora of Manouri, a Traditional Greek Whey Cheese, During Storage. International Journal of Dairy Technology, 54: 100-106.
- Ming, X., M.A. Daesche,l and Georgnaras et.al, 1993. Nisin Resistance of Foodborne Bacteria and the Specific Resistance Response of *Listeria monocytogenes*, J.N. Sofos, Scott A. Journal of Food Protection, 56: 944-948.
- Munoza, A., S. Ananoua, A. Galvezc, M. Martınez-Buenoa, A. Rodrıguezd, M. Maquedaa and E. Valdiviaa, 2007. Inhibition of *Staphylococcus aureus* in Dairy Products by Enterocin AS-48 Produced *in situ* and *ex situ*: Bactericidal Synergism with Heat. International Dairy Journal, 17: 760-769.
- Nettles, C.G. and S.F. Barefoot, 1993. Biochemical and Genetic Characteristics of Bacteriocins of Food-Associated Lactic Acid Bacteria. Journal of Food Protection, 56: 338-346.
- Oguntoyinbo, F.A., A.I. Sanni, C.M.A.P. Franz and W.H. Holzapfel, 2007. Phenotypic Diversity and Technological Properties of *Bacillus subtilis* Species Isolated from Okpehe, a Traditional Fermented Condiment. World Journal of Microbiology and Biotechnology, 23: 401-410.
- O'Sullivan, L., R.P. Ross and C. Hill, 2002. Potential of Bacteriocin-Producing Lactic Acid Bacteria for Improvements in Food Safety and Quality. Biochimie, 84: 593-604.
- Sable, S., A. Pons, S. Gendron-Gaillard and G. Cottenceau, 2000. Antibacterial Activity Evaluation of Microcin J25 Against Diarrheagenic *Escherichia coli*. Applied and Environmental Microbiology, 66: 4595-4597.
- Samelis, J., G.K. Bedie, J.N. Sofos, K.E. Belk, J.A. Scanga and G.C. Smith, 2005. Combinations of Nisin With Organic Acids or Salts to Control *Listeria monocytogenes* on Sliced Pork Bologna Stored At 4^{o}C in Vacuum Packages. Lebensmittel-Wissenschaft und-Technologie, 38: 21-28.
- Samelis, J., A. Kakouri, K.J. Rogga, I.N. Savvaidis and M.G. Kontominas, 2003. Nisin Treatments to Control *Listeria monocytogenes* Post-Processing Contamination on Anthotyros, a Traditional Greek Whey Cheese, Stored at 4 ^{o}C in Vacuum Packages. Food Microbiology, 20: 661-669.
- Settanni, L. and A. Corsetti, 2008. Application of Bacteriocins in Vegetable Food Biopreservation. International Journal of Food Microbiology, 121: 123-138.
- Settanni, L. and G. Moschetti, 2010. Non-Starter Lactic Acid Bacteria Used to Improve Cheese Quality and Provide Health Benefits. Food Microbiology, 27: 691-697.
- Thapa, N., J. Pal and J.P. Tamang, 2004. Microbial Diversity in Ngari, Hentak and Tungtap, Fermented Fish Products of North East India. World Journal of Microbiology and Biotechnology, 20: 599-607.

- Tzanetakis, N. and E. Litopoulou-Tzanetaki, 1992. Changes in Numbers and Kinds of Lactic Acid Bacteria in Feta and Teleme, Two Cheese from Ewe's Milk. Journal of Dairy Science, 75: 1389-1393.
- Ukuku, D.O. and L.A. Shelef, 1997. Sensitivity of Six Strains of *Listeria monocytogenes* to Nisin. Journal of Food Protection, 60: 867-869.
- Veljovic, K., D. Fira, A. Terzic- and Vidojevic, H. Abriouel, A. Galvez and L. Topisirovic, 2009. Evaluation of Antimicrobial and Proteolytic Activity of Enterococci Isolated From Fermented Products. European Food Research and Technology, 230: 63-70.