Research Article **Microbiology**

International Journal of Pharma and Bio Sciences

ISSN 0975-6299

INVITRO ANTIBACTERIAL ACTIVITY OF LACTOBACILLUS PLANTARUM ISOLATED FROM SOY MILK

P.SRINIVASAN*[1], K. ASSALAM KHAN[1], U.RAJA PERUMAL[1], R. VENESH KUMAR[1], K. SUGANYA[1] AND M. RAJALAKSHMI[2]

[1]Department of Microbiology, S.R.M College of Arts and Science, Chennai.
[2]Department of Microbial Biotechnology, Bharathiyar University, Coimbatore.

ABSTRACT

The aim of this study was to determine the *Lactobacillus plantarum* isolated from soy milk, the antimicrobial activity of the bacteriocin produced by the isolate against the pathogenic bacteria and antibiotic resistance of these isolates.The identity of the culture was based on characteristics of the strains of *Lactobacillus spp.* as presented in Bergey's Manual of Determinative Bacteriology, carrying out microscopy (morphology), Gram staining, growth at 15 and 45°C, and fermentation of different carbon sources and growth in 7.5% NaCl. On the basis of all of the identification tests, among fifty three isolates, twenty nine strains isolated from the soymilk were identified as *Lactobacillus sps*. A total of five among twenty nine were *L. plantarum* and showed good inhibitory activity against the pathogens. The *L. plantarum* isolates were resistant to one of the antibiotic discs used in this study. Culture supernatants obtained from the isolates of *Lactobacillus spp.* exhibited varying degrees of inhibitory activity against strains of *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeroginosa*, Enterobacter cloacae*, Bacillus subtilis*, *Klebsiella pneumoniae*, *Salmonella typhimurium* and *Salmonella typhi*

KEYWORDS; ***Lactobacillus plantarum*, Plantaricin, Antimicrobial effect, Antibiotic resistance.**

P.SRINIVASAN
Department of Microbiology, S.R.M College of Arts and Science, Chennai

***Corresponding author**

INTRODUCTION

Probiotic cultures have been associated historically with milk and dairy products, from which there is substantial evidence for positive effects on human health and general well-being.[1] The increasing demand for high quality and safe, processed food has created a niche for good quality of probiotic food.[2] Historically, *Lactobacilli* had been found to be associated with food. The food containing *Lactobacillus* are thought to be safe.[3] L*actobacillus* is an important organism recognized for their fermentative ability as well as their health and nutritional benefits.[4]

An increase in stress and modern day life, which makes a consequential demand on the immune system, can disrupt homeostasis in the gut. Similarly the direct effects of a change in dietary patterns and eating habits can effect overall gut functionality. This has led to the susceptibility of humans to various kinds of stress related and antibiotic related diseases. This calls for the use of probiotics."A probiotic is defined as a live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance".[5]

Lactic acid bacteria (LAB) have been used successfully, with few adverse effects, to prevent antibiotic associated diarrhea, to treat acute infantile diarrhea and recurrent *Clostridium difficile* disease and to treat various diarrheal illnesses.[6–8] The antagonistic property is attributed to the lowered pH, the undissociated acids and production of other primary and secondary antimicrobial metabolites produced by LAB. The metabolites produced by the fermentation process, except the volatile ones, are kept in the foods and result in growth inhibition of food spoilage or poisoning bacteria and detoxification of noxious compounds of plant origin.[9, 10]

In addition LAB produce various antimicrobial compounds, which can be classified as low-molecular-mass (LMM) compounds such as hydrogen peroxide (H_2O_2), carbon dioxide (CO_2), diacetyl (2,3-butanedione), uncharacterized compounds, and high-molecular-mass (HMM) compounds like bacteriocin.[11–14]Most of the probiotic foods are incorporated with LAB in concentrated forms such as 10^{10} cfu/g. These concentrates are usually freeze dried, spray dried or microencapsulated. These lactobacilli are typically incorporated in fermented milks,[15–18] cheeses,[19–25] and ice creams.[26]

Being the nutritious food among the indigenous foods, soy milk proves to be a good probiotic and a good source of nutrition for malnourished people. It also contains a variety of microbial load which proves to be beneficial like other probiotic bacteria. So this is being used in the process of isolation of *L. plantarum.* Soy is rich in protein, unsaturated fatty acid, lecithin, and iso flavones, contain no cholesterol or lactose and may be consumed by those suffering from the lactose intolerance. The aim of this work was to determine the strains of *Lactobacillus spp*. isolated from soy milk and to determine the antimicrobial activity and antibiotic resistance of these isolates.

MATERIALS AND METHODS

(i) Total mesophilic aerobic bacteria in food samples

About 25 ml of soy milk sample was taken aseptically and transferred to sterile plastic bags and then mixed with 225 ml of sterile buffered peptone water (BPW). Five 10-fold dilutions of this mix was then prepared and these were inoculated on plates of Nutrient Agar (Himedia).

(ii) Isolation and phenotypic characterization

About 25 ml of soy milk sample was taken aseptically. It was then transferred to sterile plastic bags and then mixed with 225 ml of sterile buffered peptone water (BPW). Five 10-fold dilutions of this mix was then prepared and these were inoculated on plates of MRS agar (Oxoid), acidified with glacial acetic acid to pH 5.5 ± 0.2 and incubated anaerobically for 48 h at 35°C.Colonies with typical characteristics were randomly selected from plates and tested

for Gram stain, cell morphology, catalase and oxidase reaction before further sugar fermentation and characterization tests.[27] During the test the cultures were kept in MRS agar stabs at refrigeration temperature.

(iii) Biochemical characterization and presumptive Identification

Growth at 8°C and 15°C in tubes containing MRS broth, growth in 7.5% NaCl, and fermentation of carbohydrates were determined as described by Schillinger and Lucke[28] and Sneath et al.[29] The carbohydrates tested were D(+) cellobiose (Difco), D(+) galactose (Difco), inulin (Difco), lactose (Difco), fructose (Difco), maltose l-hydrate (Difco), D(+) mannitol (Difco), D(+) melezitose (Difco), melibiose (Difco), D(-) raffinose (Difco), rhamnose (Difco), ribose (Difco), sorbitol (Difco), D(+) trehalose (Difco), D(+) xylose (Merck), and glucose (Difco) and sterile water were used as positive and negative controls. Gas production from glucose, dextran production from saccharose and hydrolysis of arginine were tested in MRS broth without glucose and meat extract but containing 0.3% arginine and 0.2% sodium citrate replacing ammonium citrate. Ammonia was detected using Nessler's reagent as described by Schillinger and Lucke[28] with the exception of adding glucose to the final concentration of 0.3 g/l to test NH_3 production from arginine. Production of acetoin was detected by the Voges-Proskauer test.[30]

(iv) Determination of antibiotic resistance of the isolates

In this study, four antibiotic discs were used to determine the antibiotic resistance of Lactobacillus strains. These antibiotic discs (Himedia) were as follows, ampicillin (10 µg), vancomycin (30 µg), oxacillin (1 µg),and tobramycin (10 µg). Activated cultures were grown on slopes and the bacterial cells were removed from the surface with saline. Cell suspensions (0.5 on the McFarland scale) were inoculated to Mueller-Hinton agar plates (Oxoid) containing horse blood and glucose.[31]

(v) Antibacterial activity of the strains of Lactobacillus spp. isolates

Antimicrobial effects of the presumptive strains of *Lactobacillus spp.* on *E. coli*, *S. aureus*, *P. aeroginosa*, *B. subtilis*, *K. pneumoniae*, *S. typhimurium*, E. cloacae were determined by the agar diffusion method.[32]The test bacteria were obtained from the Microbiology department of SRM medical college and research center, Chennai. The test bacteria were incubated on suitable broth media at appropriate temperature for 24–48 h. For the detection of antibacterial activity of the isolates of *Lactobacillus spp.,* MRS media containing only 0.2% glucose (MRS-0.2) was used. Ten milliliters of broth was inoculated with each isolate of *Lactobacillus spp.* and were incubated at 35°C for 48 h. After incubation, a cell-free solution was obtained by centrifuging (6000 X g for 15 min) the culture, followed by filtration of the supernatant through a 0.2 µm pore size (Himedia) cellulose acetate filter. A fraction of the supernatants were neutralized with 1N NaOH to pH 6.5 ± 0.2, and the inhibitory effect of the hydrogen peroxide was eliminated by the addition of catalase (5 mg/ml). Un-neutralized (general inhibitory effect) and neutralized (bacteriocin and bacteriocin-like metabolites) supernatants of the strains of *Lactobacillus spp*. were checked for antibacterial activity against pathogenic bacteria in inoculated nutrient agar.[9]

(vi) Thermal and pH stability of bacteriocin

Bacteriocin containing samples were incubated for 15 min at temperatures ranging from 40–100°C with 10°C increase at each point, and also autoclaved (121°C; 15 min). After heat treatment, the samples were cooled to room temperature and the remaining bacteriocin activities were determined. Bacteriocin preparations were also heated in a boiling water bath. Samples were taken at 0, 10, 30, 60 and 120 min intervals and bacteriocin activity assayed. To test the effect of pH on bacteriocin activity, the pH of bacteriocin samples was adjusted stepwise from 1–12, in steps of one pH unit, by using 1N HCl or 1N

NaOH. Samples were incubated for 1 h at 30°C and bacteriocin activity was determined.

(vii) Partial purification of bacteriocin[33]

Ammonium sulphate precipitation and dialysis: About 20 ml of the crude bacteriocin sample was taken in a glass beaker and 60% ammonium sulphate solution was added gradually on the sample. The mixture was stirred for 2 h at 4ºC using a magnetic stirrer. The solution was then centrifuged at 10,000 rpm for 20 min at 4°C. The supernatant was discarded and the precipitate was re-suspended in 25 ml of 10mM potassium phosphate buffer (pH 7.0).The dialysis membrane having a cut off value not more than 1200 kDa was cut to a required length and was opened with gentle squeezing along with gradual addition of distilled water.One end of the membrane was closed by tying the end with a thread. The bacteriocin sample was poured on to the dialysis membrane and was tied on to a glass rod.It was kept half way immersed in a beaker containing 10mM phosphate buffer solution and then mixed slowly using a magnetic stirrer for 12–18 h.During the mixing process the buffer was changed for every 3 h to ensure proper dialysis of the protein.After the dialysis the membrane bag was carefully collected and stored at 4°C. The antibacterial activity of the dialyzed bacteriocin was determined using the agar well diffusion method. The amount of the protein present in the samples were quantified using the Lowry's method.

RESULTS

Twenty nine presumptive *Lactobacillus* were isolated from the four soy milk samples. All the isolates were catalase negative, Gram positive, oxidase negative rods producing no gas from glucose. On the basis of the identification procedures, five were identified as *L. plantarum*.

The total number mesophilic aerobic bacteria present in the soy milk samples were 4.9, 5.2, 5.5 and 5.4 log CFU/g, respectively. Metabolic characteristics and presumptive identification *Lactobacillus plantarum* isolated from the four soy milk samples are shown in the **Table 1**.

Table 1
Metabolic characteristics and presumptive identification of L. plantarum isolated from four soy milk samples

Presumptive identification	No of isolates	Morphology	Growth at				NH_3 from arginine	Sugar fermentation												
			15°C only	45°C only	Growth at 15 and 45°C	from glucose		Cellobiose	Mannitol	Mannose	Melebiose	Raffinose	Ribose	Salicin	Lactose	Rhamnose	Sorbitol	Xylose	Trehalose	Arabinose
L. plantarum	Lp1	S	+	−	+	−	−	+	+	+	+	+	+	+	−	−	−	−	−	−
	Lp2	S	+	−	+	−	−	±	+	+	+	+	+	+	−	−	−	−	−	−
	Lp3	S	+	−	+	−	−	+	+	±	+	+	+	+	−	−	−	−	−	−
	Lp4	S	+	−	+	−	−	±	+	+	+	+	+	+	−	−	−	−	−	−
	Lp5	S	+	−	+	−	−	+	+	+	+	+	+	±	−	−	−	−	−	−

± intermittent in sugar fermentation, S- smooth colony

The antibiotic resistance of the isolates is shown in the **Table 2.** All the isolates were resistant to the tobramicin antibiotic and were susceptible to the other antibiotics used in the study.

Table 2
Determination of the antibiotic resistance of the isolates

Isolates	Ampicillin (10 µg)	Tobramicin (10 µg)	Vancomycin (30 µg)	Oxacillin (1 µg)
Lp1	9.8	–	10.6	11.1
Lp2	10.2	–	9.3	10.2
Lp3	10	–	10.4	10.1
Lp4	10.8	–	11.4	9.6
Lp5	10.5	–	11	11.1

The antibacterial activities exhibited by *L. plantarum* are presented in **Table 3**. Culture supernatants obtained from *L. plantarum* isolates exhibited varying degrees of inhibitory activity against strains of *E. coli*, *S. aureus*, *P. aeroginosa*, *B. subtilis*, *K. pneumoniae*, *S. typhimurium*, *S. typhi* and *E. cloacae*.

Table 3
Antibacterial activity of the L. plantarum bacteriocin (crude) against the test bacteria

Isolate no	Zone of inhibition (in mm)							
	E. coli	*S. aureus*	*P. aeroginosa*	*B. subtilis*	*K. pneumoniae*	*S. typhimurium*	*E. cloacae*	*S. typhi*
Lp1	21.9	19.2	20.8	–	20.1	21.3	22.8	15.6
Lp2	20.5	21.2	20.5	–	20.3	21.4	20.4	14.5
Lp3	21.4	22.8	22	–	20.5	21.9	21.1	13.5
Lp4	22.1	22.7	21.6	–	21.5	20.2	21.3	14.3
Lp5	22.5	23.4	23.6	–	22.2	22.9	21.9	18.2

The antibacterial activities of the partially purified bacteriocin isolated from *L. plantarum* isolates are presented in **Table 4** (Fig 1). Partially purified culture supernatant exhibited varying degrees of inhibitory activity against strains of *E. coli*, *S. aureus*, *P. aeroginosa*, *B. subtilis*, *K. pneumoniae*, *S. typhimurium*, *S. typhi* and *E. cloacae*.

Table 4
Antibacterial activity of the L. plantarum bacteriocin (partially purified) against the pathogens

Isolate no.	Zone if inhibition (in mm)							
	E. coli	*S. aureus*	*P. aeroginosa*	*B. subtilis*	*K. pneumoniae*	*S. typhimurium*	*E. cloacae*	*S. typhi*
Lp1	21	18.4	19.7	–	19.4	19	22.7	13
Lp2	19.8	19.2	20.3	–	19.1	19.3	19.5	11.5
Lp3	20.6	22.6	22	–	20	20	21	11
Lp4	22	21.8	21.4	–	21.2	19.5	21.2	12.6
Lp5	22.1	23	23.4	–	21	22.4	21.2	13.3

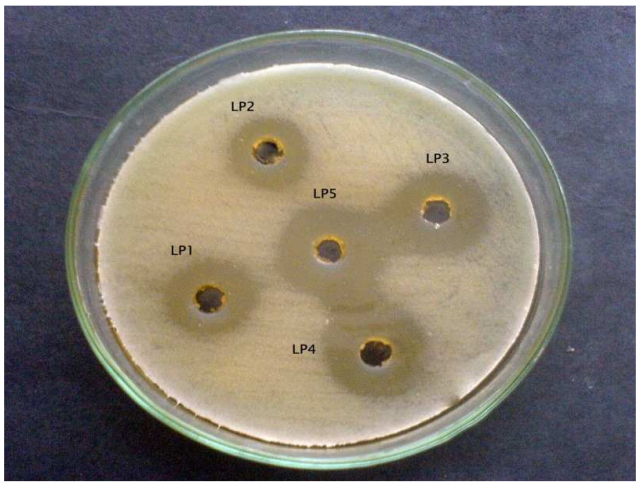

Figure 1
Inhibitory activity of the partially purified bacteriocin samples on E. coli. Highest zone of inhibition was shown by LP5 isolate.

The bacteriocins were observed for their ability at different pH and temperature. In the Fig 2 bacteriocins of all the isolates retained their activity at mean pH range between 3 and 11. The bacteriocin samples of all the isolates retained their antibacterial activity at a temperature up to 80°C for 30 min which is evident from the Fig 3.

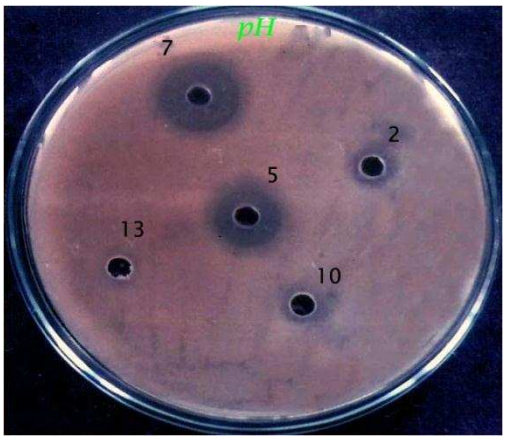

Figure 2
Effect of pH on the partially purified bacteriocin samples. Zone of inhibition was seen on the samples with pH 5 and 7.

Figure 3
Effect of temperature on the partially purified bacteriocin samples.

The quantification of the protein present in the partially purified samples was performed using the Lowry's method. The amount of the protein present in the partially purified samples are presented in the following table.

Table 5
Protein quantification of the partially purified bacteriocin samples

Bacteriocin sample of isolate no.	Amount of protein µg/ml
Lp1	34
Lp2	48
Lp3	36
Lp4	42
Lp5	52

DISCUSSIONS

From a total of 29 presumptive isolates five of the isolates (Lp1–Lp5) were found to be *L. plantarum*. All the five isolates of *L.plantarum* showed strong antibacterial activity (≥ 20 mm) against *E. coli*, *S. aureus*, *P. aeroginosa*, *K. pneumoniae*, *S. typhimurium*, and *E. cloacae*. However the antibacterial effect against *S. typhi* was less (≤ 20 mm) when compared to the other pathogens. The isolates didn't show any antibacterial activity against *B. subtilis*. The antibacterial activity exhibited by the Lp5 isolate was higher when compared to the activity of the other isolates.Partially purified bacteriocin samples exhibited good inhibitory effect against all the pathogens. Here also we observed that the partially purified bacteriocin of the Lp5 isolate to show a good antibacterial activity against all the pathogens. But there was no activity exhibited by the bacteriocin against the *B. subtilis*. Schillinger and Lucke[9] and Toksoy et al.[34] reported that some *L. plantarum* and *L. sake* strains from meat and meat products had inhibitory effects against several bacteria. In addition, Xanthopoulos et al.[35] indicated that *L. paracasei* subsp. *paracasei* and *L. acidophilus* strains isolated from infant feces had weak antibacterial activity on *E. coli* and *Yersinia enterocolitica*. The antimicrobial effect exerted by LAB is the production of lactic acid and reduction of pH, and acetic acid, diacetyl, hydrogen peroxide, fatty acids, aldehydes and other compounds.[11]Aroutcheva et al.[36]revealed that no correlation was found between bacteriocin activity, lactic acid and hydrogen peroxide production. They found that three Lactobacillus strains produced H_2O_2 but did not demonstrate any inhibitory effect. Yuksekdag et al.[37] reported that *Lactococcus lactis* subsp. *cremoris* Z20S strain produced maximum lactic acid but did not produce H_2O_2. Moreover, the strain had an inhibitory effect against *S. aureus* but no inhibitory effect against *E. coli* and *P. aeruginosa*. Alexandre et al.[38] reported that 192 strains of lactic acid bacteria were isolated from five samples of Artisanal minas cheese. The results of direct inhibition test indicated that 48 strains inhibited the in vitro growth of the indicator microorganisms *S. aureus* and *Listeria monocytogenes*. In a study by Tadesse et al.[39], LAB involved in the fermentation of traditional beverages had an antimicrobial property against various food-borne pathogens and the inhibitory products were extracellular and diffusible. The observed inhibitory property of LAB was influenced by the medium they grew in. Many LAB are resistant to antibiotics. This resistancesattributes are often intrinsic and nontransmissible.[40] On the other hand, intrinsically antibiotic-resistant probiotic strains may benefit patients whose normal intestinal microbiota has become unbalanced or greatly reduced in numbers due to the administration of various antimicrobial agents.[41] Among antibiotic resistances, vancomycin resistance is of major concern because vancomycin is one of the last antibiotics broadly efficacious against clinical infections caused by multidrug resistant pathogens.[42] All the isolates were sensitive to the vancomycin in the study.Vancomycin resistance was a general characteristic of bifidobacteria.[40]

Gilliand[4] studied the bacteriocin produced by *Lactobacillus plantarum*, which were partially purified by ammonium sulphate precipitation and dialysis. Bacteriocin activity

was determined against various pathogens. In this study the bacteriocin produced by the all the five isolates of *L. plantarum* after partial purification showed a strong antibacterial activity against the candidate pathogenic microorganisms. In addition the partially purified bacteriocin of the Lp5 isolate had higher antibacterial activity than the samples of the other isolates.

CONCLUSION

In conclusion, the elucidation of the molecular genetics of autochthonous LAB, isolated from specific food sources like soy milk gives an opportunity to a make genetically defined collection of natural isolates of LAB. Such a collection could be used for construction of specific starter cultures for fermented food products. Starter cultures prepared in that way

could be used for production of fermented products with geographical origin. Natural isolates of LAB that produce well characterized bacteriocins could be used for food preservation and increasing the shelf lifeof the product. On the other hand, genetically well characterized natural isolates of LAB could be eventually a source of genes for construction of new starter cultures or for the improvement of existing ones by using genetic engineering. Further studies have to be done in characterizing the bacteriocin produced by the *L. plantarum* isolates got from the soy milk source. In addition ultra-purification techniques and proteomic studies would help in the gaining a deeper understanding about the bacteriocin produced by the isolates and their effectiveness. Finally, bearing in mind the increased interest for dairy products containing specific bacterial species with potential health-improving properties, isolation of LAB from humans that could be used as probiotics is also extremely interesting. Such isolates could be a basis for construction of starter cultures for the production of novel and functional foods.

AKNOWLEDGEMENT

We, on the whole thank Dr. V. Brindha Priyadharshini, Proffessor, Department of Microbial Biotechnology, Bharathiyar University, Coimbatore, for her everlasting support which made this project a successful one. We also extend our sincere thanks to Mrs. K. Abirami, Assistant Professor, Department of Microbiology, SRM Arts and Science College, Chennai, for her encouragement and timely help.

REFERENCES

1. Klaenhammer TR., Probiotic bacteria: today and tomorrow. J Nutrition, 130(2): 415–416, (2000)
2. Sakala R.M., Hayashidani H., Kato Y., Kaneuchi C., and Ogawa M., Isolation and characterization of *Lactococcuspiscium* strains from vacuum packaged refrigerated beef. J Applied Microbiology, 92:173–179, (2002)
3. Adams M.R., Marteau P., On the safety of Lactic acid producing bacteria. Int J Food Microbiol, 27:263–264, (1995)
4. Gilliand S.E., Sanni A.I., Onilude A., Ogunbanwo S.T., and Smith SI., Antagonistic activity of bacteriocin produced by Lactobacillus species from ogi, an indigenous fermented food. J Basic Microbiol, 20(3): 189–195, (1999)
5 Salminen S., Ouwehand A.C., Isolauri E., Clinical application of probiotic bacteria. Int J Dairy Food Sci, 8: 563–572, (1998)
6. Siitonen S., Vapaatalo H., Salminen S. et al., Effect of Lactobacillus GG yogurt in prevention of antibiotic associated diarrhea. Annals Med, 22: 57–59, (1990)
7. Saavedra J.M., Bauman N.A., Oung I. et al., Feeding of *Bifidiobacteriumbifidum* and *Streptococcus thermophilus* toinfants in hospital for the prevention of diarrhea and shedding ofrotavirus. Lancet, 344: 1046–1049, (1994)
8. Biller J.A., Katz A.J., Floves A.F. et al., Treatment of recurrentClostridium difficile colitis with Lactobacillus GG. JPediatric Gastroenterology Nutrition 21: 224–226, (1995)
9 Schillinger U., Lucke F.K., Antimicrobial activity of *Lactobacillus sake* isolated from meat. App Environ Microbiol, 55:1901–1906, (1989)
10 S. Salminen, E. Isolauri and E. Salminen. Clinical uses of probiotics for stabilizing the gut mucosal barrier: successful strains and future challenges. In: G. Venema, J. H. J. HuisinÕt veld, J. Hugenholtz (eds.), *Lactic acid bacteria: genetics, metabolism and applications,* Kluwer Academic Publishers, Netherlands, 1996, pp. 251-260.

11. Jay JM., Antimicrobial properties of diacetyl. AppEnviron Microbiol, 44: 525–532, (1982)
12. Klaenhammer TR., Bacteriocins of lactic acid bacteria. Biochimie,70: 337–349, (1988)
13. Piard J.C.,Desmazeaud M., Inhibiting factors produced by lacticacid bacteria: 1. Oxygen metabolites and catabolism endproducts.Lait, 71 (5): 525–541, (1991)
14. Piard J.C., Desmazeaud M., Inhibiting factors produced by lacticacid bacteria: 2. Bacteriocins and other antibacterial substances.Lait, 72: 113–142, (1992)
15. Nighswonger B.D., Brashears M.M., Gilliland S.E., Viability of*Lactobacillus acidophilus* and *Lactobacillus casei* in fermented milk products during refrigerated storage. JDairy Sci, 79: 212–219, (1996)
16. Sanders M.E., Walker D.C., Walker K.M. et al., Performance ofcommercial cultures in fluid milk applications. J DairySci, 79: 943–955, (1996)
17. Heller K.J., Probiotic bacteria in fermented foods: productcharacteristics and starter organisms. American J ClinicalNutrition, 73: 374S–379S, (2001)
18. Oliveira M.N., Sodini I., Remeuf F. et al., Effect of milksupplementation and culture composition on acidification, texturalproperties and microbiological stability of fermented milkscontaining probiotic bacteria. IntDairy J, 11:935–942, (2001)
19. Gomes A.M., Malcata F.X., Development of probiotic cheesemanufactured from goat milk: response surface analysis viatechnological manipulation. J Dairy Sci, 81: 1492–1507, (1998)
20. Stanton C., Lynch P.B., Collins J.K. et al., Probiotic cheese.Int Dairy J, 8: 491–496, (1998)
21. Vinderola C.G., Prosello W., Ghiberto T.D. et al., Viability of probiotic (*Bifidobacterium, Lactobacillus acidophilus and Lactobacillus casei*) and non probioticmicroflora in Argentinian Fresco cheese. J Dairy Sci, 83: 1905–1911, (2000)
22. Barraquio V.L., Militante S.T., Gonzaga J.O. et al., Fermented milk drink as starter adjunct in the manufacture of probiotic white softcheese. Philippine Agriculture Scientist, 84: 382–387, (2001)
23. Nayra S., Sharaf O.M., Ibrahim G.A., Incorporation and viability of some probiotic bacteria in functional dairy food: I. Soft cheese.Egyptian J Dairy Sci, 30: 217–229, (2002)
24. Perko B., BogovicMatijasic B., Rogelj J., Production of probiotic cheese with addition of *Lactobacillus gasseri*LF221(Rif) and K7(Rif). ZbornikBiotehniskeFakulteteUniverze v Lubljani.Kmetijstvo, Zootehnika, 80: 61–70, (2002)
25. Suarez-Solis V., Cardoso F., Nunez de Villavicencio M. et al.,Probiotic fresh cheese. Alimentaria, 39: 83–86, (2002)
26. Christiansen P.S., Edelsten D., Kristiansen J.R., Some properties of ice cream containing *Bifidobacteriumbifidum* and *Lactobacillusacidophilus*. Milchwissenschaft, 51: 502–504, (1996)
27. W. Harrigan, M McCance, Ed. Laboratory Methods in Food and Dairy Microbiology, 8th Edn. Academic Press, London, UK: (1990)
28. Schillinger U., L.cke FK., Identification of lactobacilli from meat and meat products. Food Microbiol, 4:199–208, (1987)
29. P. H. Sneath, N. S. Mair, M. E. Sharpe et al. Bergey's Manual of Systematic Bacteriology, Vol 2, Williams and Wilkins, Baltimore: 1599, (1986)
30. Reuter G., Laktobazillen und engverwandteMikroorganismen in Fleisc und Fleischerzeugnissen 2. Mitteliung: Die Charakterisierung der isoliertenLaktobazillen-St.mme.Fleischwirtschaft, 50: 954–962, (1970)
31. Bauer A.W., Kirby W.M.M., Sheris J.C.etal., Antibiotic susceptibility testing by a

standardized single disk method. American J ClinPathol, 45: 493–-496, (1996)
32. Fleming H.P., Etchells J.L., Costilow R.L., Microbial inhibition by an isolate of Pediococcus from cucumber brines. AppMicrobiol, 30: 1040–1042, (1985)
33. Ogunbanwo S.T., Sanni A.I., Onilude A., Characterization of bacteriocin produced by *Lactobacillus plantarum* F1 and *Lactobacillus brevis* OG1. African J Biotechnol, 2(8), 219–227, (2003)
34. Toksoy A., Beyatlõ Y., Aslõm B. Studying on metabolic and antimicrobial activities of some *L. plantarum* strains isolated from sausages. Turkish J Veterinary Animal Sci, 23: 533–540, (1999)
35. Xanthopoulos V., Litopoulou-Tzanetaki E., Tzanetakis N., Characterization of Lactobacillus isolates from infant faeces as dietary adjuncts. Food Microbiol, 17: 205–215, (2000)
36. Aroutcheva A., Gariti D., Simon M. et al., Defense factors of vaginal lactobacilli. American JObstetrics Gynecology, 185: 375–379, (2001)
37. Yuksekdag Z.N., Beyatli Y., Aslim B., Determination of some characteristics coccoid forms of lactic acid bacteria isolated from Turkish kefirs with natural probiotic. Lebensmiteel und Wiss. u.Technology, 37: 663–667, (2004)
38. Alexandre D.P., Silva M.R., Souza M.R. et al., Antimicrobial activity of lactic acid bacteria from artisonal minas cheese against indicator microorganisms. ArquivoBrasilerio de MedicinaVeterinaria e Zootecnia, 54: 424–428, (2002)
39 . Tadesse G., Ephraim E., Ashenafi M., Assessment of the antimicrobial activity of lactic acid bacteria isolated from Borde and Shamita, traditional Ethiopian fermented beverages, on somefood borne pathogens and effect of growth medium on the inhibitory activity. Int J Food Safety, 5: 13–20, (2005)
40. Curragh H.J., Collins M.A., High levels of spontaneous drug resistance in Lactobacillus. J App Bacteriology, 73: 31–36, (1992)
41. Salminen S., von Wright A., Morelli L. et al., Demonstration of safety of probiotics-a review. Int J Food Microbiol, 44: 93–106, (1998)
42. Johnson A.P., Uttley A.H.C., Woodford N. et al. Resistance to vancomycin and teicoplanin: an emerging clinical problem. Clinical Microbiol, 3: 280–291, (1990)