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Plasmid Profiling and Curing of *Lactobacillus* Strains Isolated from Fermented Milk for Probiotic Applications

B. Lavanya, S. Sowmiya, S. Balaji and B. Muthuvelan
School of Bio Sciences and Technology (SBST), VIT University, Vellore-632 014, India

Abstract: In this study, the antimicrobial susceptibilities and presence of plasmids in 7 probiotics strains which had been isolated from the fermented milk were determined. Resistance to 8 commonly used antibiotics β- lactans (penicillin, ampicilin), gram positive spectrum (vanomycin), broad spectrum (rifampin, trimethoprim) and aminogycosides (kanamycin, streptomycin, and bacitracin) was assessed by disk diffusion. Among these strains 20, 20, 60, 70, 90 and 100% were found to be exhibit a significant degree of resistance to kanamycin, trimetroprim, rifampicin, kanamycin, amphicilin and penicillin respectively. Further, plasmid profile and curing of plasmid were performed for the seven isolates. Analysis of the plasmid profiles of the 7 cured derivatives revealed loss of plasmids except 2 strains where curing was partially effective. All the strains lost penicillin resistance after curing indicating that plasmids encodes for resistance character. However, vanomycin resistance is not lost upon curing which indicates that such resistance is usually intrinsic (chromosomally encoded and not transmissible). Finally, the antimicrobial susceptibility after curing was done to check the safety aspect of the isolates for their application as probiotics and among the 7 strains, 5 were proved to be potent probiotics.

Key words: Food and diary markets, *Lactobacillus* spp., plasmid profiling, probiotics

INTRODUCTION

Probiotics are commonly defined as viable micro organisms that exhibit a beneficial effect on the health of the host when they are ingested (Fuller, 1989; Perdigon *et al*., 1990). Mainly, the ability to reduce serum cholesterol levels, antimicrobial substrate production and immune modulation are considered as effective properties. In which, *Lactobacillus* (Lactic Acid Bacteria, LAB) is a commercially important bacterium with wide variety of application, both in the food industry and as a probiotic agent for the improvement of human health (Arunachalam *et al*., 2000; Fuller, 1989; Grahn *et al*., 1994). Most LAB strains regardless of their source harbor at least one indigenous plasmid and often more (Ghosh *et al*., 2000; Posno *et al*., 1991; Pouwels and Leer, 1993). In which, some LAB may carry potentially transmissible plasmid encoded antibiotic resistance genes (Ahn *et al*., 1992; Ishiwa and Iwata, 1980; Lin *et al*., 1996) and any strains harboring antibiotic resistance plasmids are considered unsuitable for use as human or animal probiotics (Kalavathy *et al*., 2003; Ghosh *et al*., 2000). However, the importance of intrinsic antibiotic resistant strains which may benefit patients whose normal intestinal micro biota has become unbalanced or greatly reduced in numbers due to administration of various antimicrobial agents have also been reported (Billman-Jacobe, 1996; Kullen and Klaenhammer, 1999; Posno *et al*., 1991).

Further, some of these plasmids have important characteristics such as drug resistance, metabolic functions, a restriction system or phage resistance and some plasmids are important for industrial applications (Adams and Marteau, 1995; Cebeci and Gürakan, 2003; Gill *et al*., 2001b; Saarela *et al*., 2000). In addition, most of the natural isolates carried low molecular weight plasmid DNA, which may fetch interest on their application to rDNA technology. However, according to our current interest, exchange of antibiotic resistance determinants in bacteria has become a topic of major concern especially in the food, feed and medicinal industry. Hence, in this study, to further test the safety of these LAB strains which were isolated from the fermented milk in our region, we investigated their antibiotic susceptibilities after curing the plasmids to propose as potent probiotcs.

MATERIALS AND METHODS

Isolation of cultures: All the strains were isolated from fermented milk samples collected from various sources and places during the period of January 2010 to August 2010 at VIT University, Vellore, Tamil Nadu, India. The samples were diluted serially from 10^{-1} to 10^{-9} and the dilutions 10^{-4} to 10^{-9} were plated onto Man, Rogosa and Sharpe media (MRS) agar. The individual colonies with different morphology were picked using tooth pick and grown in MRS broth. Further it was plated to check for

Corresponding Author: B. Muthuvelan, Professor, School of Bio sciences and Technology, VIT University, Vellore - 632 014, Tamil Nadu, India. Tel: 91-416-2243091 Ext. 2556; Fax: 91-416-2243092

purity. These cultures were subjected to preliminary screening of LAB with Gram staining and catalase reaction. From the, 47 isolates, seven strains (*Lactobacillus pentosum* (L08), *L. Jungurthi* (L10), *L. Reuteri* (L16), *L. fermentum* (L18), *L. plantarum* (L29), *L. brevis* (L43), and *L. casei* (L47)) were selected based on their probiotic characters (Ulrich and Friedrich 1987). Glycerol stocks of the screened isolates were prepared by mixing 1 mL of 80% glycerol with 1 mL of the culture broth and stored at -20ºC. A set of MRS stabs were also made and stored at 4ºC for use as working culture. All the procedures were adapted as proposed in earlier reports (Ulrich and Friedrich, 1987; Ljungh and Wadstrom, 2006).

Plasmid profiling: Plasmid isolation: Overnight *Lactobacillus* (10 mL) culture was used for the plasmid extraction. After centrifuging at 8000 rpm for 10 min, the pellet was resuspended in 25% sucrose containing 30 mg/mL lysozyme, to a final volume of 200 µL. This was incubated for 15 min at 37 ºC and 400 µL of alkaline SDS was added and incubated for 7 min at 37ºC. Then 300 µL of ice cold sodium acetate (3M, pH 4.8) was added. After mixing well, the contents were centrifuged at 12000 rpm for 15 min and the supernatant was carefully collected to a fresh eppendorf tube and 650 µL of isoproponal was added and centrifuged at 12000 rpm for 15 min and the liquid content were discarded carefully. The pellet was resuspended with 320 µL of sterile distilled water, 200 µL of 7.5 M ammonium acetate containing 0.5 mg/mL ethidium bromide and 350 µL of phenol chloroform were added and were centrifuged at room temperature. The supernatant was taken carefully and 100% ethanol (-20ºC) was added to precipitate the DNA. The mixture was centrifuged at 12000 rpm for 15 min and the pellet was dried and resuspended in TER buffer. Finally the bands were visualized in 0.8% agarose gels with 0.5 mg/mL of ethidium bromide in 10 mM Tris-acetate buffer. This procedure was conducted according to the method described by O'Sullivan and Klaenhammer (1993) and others (Anderson and McKay, 1983; Frere, 1994).

Plasmid curing: Curing of plasmid was done (Anderson and McKay, 1983; Marcelo *et al*., 1998) by exposing the overnight grown culture to elevated temperature (37ºC) and 1% Sodium Dodecyl Sulphate (SDS). These cultures were then streaked onto MRS plates and incubated for 24 h. The colonies found were cured colonies and it was inoculated to sterile MRS broth. This culture was subjected to plasmid isolation and visualized in 0.8% agarose gels with 0.5 mg/mL of ethidium bromide in 1 mM Tris-acetate buffer.

Antibiotic susceptibility after curing: Each cured strain was inoculated into the MRS broth which was incubated at 37ºC for 12 h. Plates were made with Muller Hinton agar and allowed to solidify. The cultures were inoculated in the plates using sterile swab by spread plate technique. The antibiotic discs of kanamycin, penicillin, vancomycin, ampicillin, streptomycin, bacitracin, trimethoprim, and rifampicin were placed in the plates (Bauer *et al*., 1966; Koneman, 1997). Agar plates with antibiotic disks were then incubated for 24 h. The diameters of the inhibition zones were measured using a ruler under a colony counter apparatus (Gallenkamp, England). The results (average of five readings) were expressed as sensitive (S), marginally susceptible (I) and resistant (R) as per the recommended standards reported standards (Acar and Goldstein, 1991; Woodford *et al*., 1995)

Screening of probiotic characters: Cholesterol reduction assay: The cholesterol reduction assay was performed by methods proposed by Rudel and Moris (1973). LAB isolates were grown in MRS broth supplemented with 0.3% bile salt and 10 mg of cholesterol dissolved in 500µl of ethanol was added to 100 mL of MRS broth with bile salt. The culture was grown for 24 h at 37ºC and the cells were harvested by centrifugation at 8000 rpm for 10 min at 4ºC. The spent broth was collected and used for cholesterol assay and the uninoculated broth was considered as control. To the 1 mL of spent broth, 3 mL of 95% ethanol followed by 2 mL of 50% KOH were added. The contents were mixed well after addition of each component and the tubes were heated for 10 min at 60ºC in a water bath. After cooling, 5 mL of hexane was dispensed and vortexed for 5 min at 20 sec interval. Then the tubes were allowed to stand for 15 min at 30ºC to permit phase separation. From which, 2.5 mL of hexane layer was transferred to a fresh test tube and allowed to dry completely. Then 1.5 mL of ferric chloride reagent was added to each test tube and allowed to stand for 10 min. Finally, 1 mL of concentrated sulphuric acid was added along the sides of the tube and the mixture was vortexed and allowed to stand for 45 min at 30ºC. The optical density was measured at 540 nm and the percentage assimilation was calculated.

Screening for exopolysaccride producers: The cultures were streaked onto MRS agar plates and incubated for 24 h at 37ºC and the strains which produced slimy colonies were recorded as capable of producing exopolysaccrides (Vijayendra *et al*., 2008). The selected producers were inoculated to 10 mL of MRS broth and incubated overnight. Then it was centrifuged at 10000 rpm for 20 min and the supernatant was transferred to fresh tube and twice the volume of ice cold isopropanol was added and exopolysaccride was allowed to precipitate overnight. It was centrifuged at 12000 rpm for 30 min and the pellet was reprecipitated with isopropanol for decolourisation. Then the pellet was dissolved in 1 mL of sterile distilled

Table 1: Antibiotic susceptibility of test strains[a]

	L08	L10	L16	L 18	L29	L43	L47
β-Lactans							
Penicillin	R	R	R	R	R	R	R
Ampicilline	R	R	R	R	R	R	R
Gram+spectrum							
Vanomycin	R	S	S	R	S	R	S
Broad spectrum							
Rifampin	R	R	R	R	S	R	R
Trimethoprim	R	R	R	R	I	R	R
Aminoglycosides							
Kanamycin	S	R	I	I	R	R	R
Streptomycin	S	R	S	I	S	R	R
Basitracin	I	S	S	S	R	S	S

R, Resistant; I, marginally susceptible; S, susceptible

[a]L08: *Lactobacillus pentosum*, L10: *L. jungurthi*, L16: *L. reuteri*, L18: *L. fermentum*, L29: *L. plantarum*, L43: *L. brevis*, and L47. *L. casei*

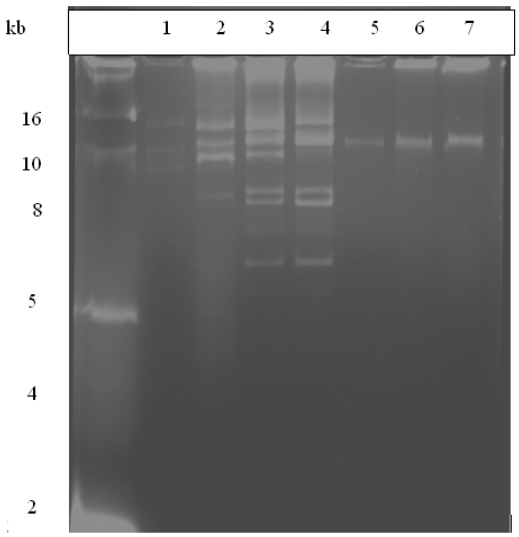

Fig. 1: Plasmids isolated from LAB strains. Lane 1: *Lactobacillus pentosum* (L08), Lane 2: *L. jungurthi* (L10), Lane 3: *L. reuteri (*L16), Lane 4: *L. fermentum* (L18), Lane 5: *L. plantarum* (L29), Lane 6: *L. brevis* (L43), and Lane 7: *L. casei* (L47)

water. And the amount of exopolysaccride was determines by phenol sulphuric acid method (Dubois *et al*., 1956; Vijayendra *et al*., 2008)

RESULTS

In the present study seven newly identified probiotic isolates were tested for antibiotic susceptibilities and the presence of plasmids, latter was attempted to determine whether strains carry any plasmid encoded antibiotic resistance genes. Results (Table 1) showed that strains L43 showed resistance to maximum of 7 antibiotics leaving bacitracin (aminoglycosides antibiotic). Among the other strains, L47 and L10 strains were observed with susceptibility to two antibiotics (bacitracin and vanomycin) and L08 and L18 showed susceptibility three antibiotics (bacitracin, kanamycin and streptomycin). The remaining two strains, L16 and L29 have been observed with maximum susceptibility to 4 antibiotics.

Table 2: Profile of plasmid from LAB strains

LAB Strains	No. of plasmids
Lactobacillus pentosum (L08)	2
L. jungurthi (L10)	4
*L. reuteri (*L16)	8
L. fermentum (L18)	8
L. plantarum (L29)	1
L. brevis (L43)	4
L. casei (L47)	1

Plasmid profile: The plasmid profiles of 7 strains are shown in Fig. 1. Plasmids ranging in size from 2.5 to 20 kb were detected in all the examined strains and the number of plasmids observed in each samples are represented in Table 2. In which L16 and L18 strains were observed with maximum number of plasmids and L 29 and L47 strains had observed with only one plasmid. Further, all strains based on the presence of a plasmid bands were subjected to clustering analysis (Fig. 2) with the help of software NTSYspc (Rohlf, 1994). In this, strain L08 has high coefficient so it's more closely related to all other strains. In rest of the strains, L29, L43 and L29 form one group and rest the rest are falling in other group.

Plasmid curing: These seven strains were consequently subjected to a plasmid curing procedure with SDS, in order to cure their respective antibiotic resistance properties (Fig. 3 and Table 3). Of these seven *Lactobacillus* strains, it was found effective to most of the strains except two strains (L18, L08), where the curing was partially efficient. The loss of resistance after curing indicates the presence of the resistance character in plasmid and such strains were rejected considering the safety of the organism. Further, the SDS failed to eliminate the plasmid even after prolonged sub culturing (every 24 h for 28 days) in sublethel concentrations. After curing, the selected five strains were studied for other

Table 3: Antibiotic susceptibility after curing

Sample	V	P	R	T	B	K	A	S
L08	R	S	R	R	I	I	R	S
L10	S	S	R	S	S	R	S	R
L16	S	S	R	R	S	I	R	I
L18	R	S	R	R	S	I	R	I
L29	S	S	S	I	R	R	S	S
L43	R	S	R	R	S	R	R	R
L47	S	S	R	R	S	R	R	R

R: Resistant; I: marginally susceptible; S: susceptible. L08: *Lactobacillus pentosum*, L10: *L. jungurthi*, L16: *L. reuteri*, L18: *L. fermentum*, L29: *L. plantarum*, L43: *L. brevis*, and L47. *L. casei*. V: Vanomycin, P: Penicillin, R: Rifampin, T: Trimethoprim, B: Bacitracin, K: Kanamycin, A: ampicillin, S: streptomycin

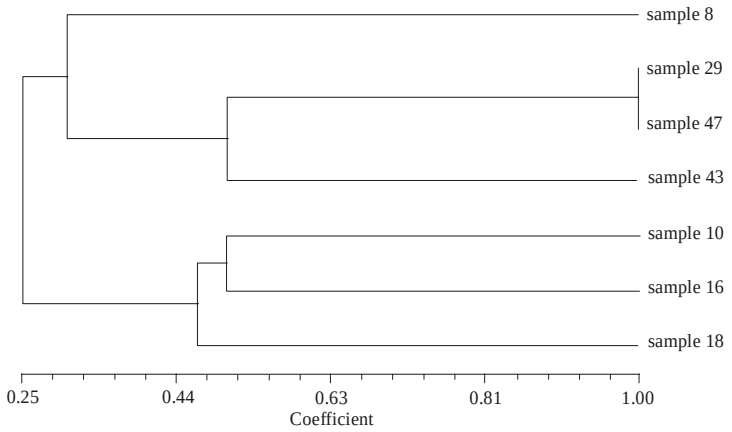

Fig. 2: Clustering of plasmid DNA from LAB isolates from fermented milk by NTSYpc software. Plasmids isolated from LAB strains. Sample 8: *Lactobacillus pentosum*, Sample 10: *L. jungurthi*, Sample 16: *L. reuteri*, Sample 18: *L. fermentum*, Sample 29: *L. plantarum*, Sample 43: *L. brevis*, and Sample 47: *L. casei*

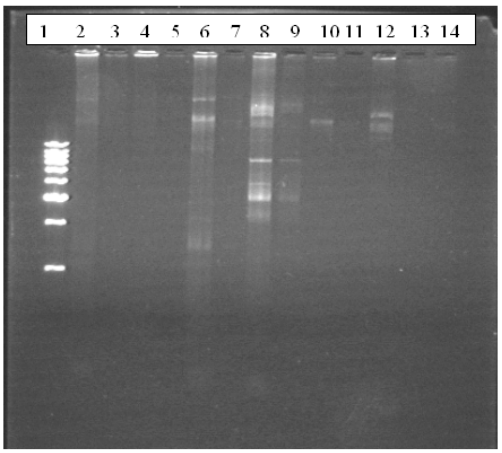

Fig. 3: Plasmids profiles of LAB strains and their cured derivatives. Lane 1: *Lactobacillus pentosum* (L08), Lane 2: L08, cured, Lane 3: *L. jungurthi* (L10), Lane 4: L10, cured, Lane 5: *L. reuteri* (L16), Lane 6: L16, cured, Lane 7: *L. fermentum* (L18), Lane 8: L18, cured, Lane 9: *L. plantarum* (L29), Lane 10: L29, cured, Lane 11: *L. brevis* (L43), Lane: L43, cured, Lane 13: *L. casei* (L47) and Lane 14: L47, cured.

Table 4: Selection of potential probiotic strains[a] after curing

Isolates	Before curing	After curing	Inference
L08	VPRTA	VRTA	*
L10	PRTKAS	RKS	-
L16	PRTA	RTA	*
L18	VPRTA	VRTA	*
L29	PBKA	BKA	-
L43	VPRTKAS	VRTKAS	*
L47	PRTKAS	RTKAS	*

*: selected; -: not selected; [a]L08: *Lactobacillus pentosum*; L10: *L. jungurthi*; L16: *L. reuteri*; L18: *L. fermentum*; L29: *L. plantarum*; L43: *L. brevis*; L47: *L. casei*

probiotic characters like cholesterol reduction & exopolysaccharide production and the results are presented in Table 4 and 5. From the overall results, it has been observed that, these five strains show significant properties to be considered as potential probiotics for further applications.

DISCUSSION

Most of *Lactobacillu*s species, regardless of their source (Plants, meat, silage, sourdough or gastrointestinal tract), harbor at least one indigenous plasmid (Pouwels and Leer, 1993). The functions of these plasmids have classically been correlated with phenotypical properties, including drug resistance, carbohydrate metabolism,

Table 5: Probiotic characters for selected strains

Isolates	Genera	Cholesterol assimilation	Exopolysaccride productivity	Resistant character
L08	*L. pentosum*	77.55	63.44	VRTA
L16	*L. reuteri*	83.33	95.20	RTA
L18	*L. fermentum*	78.57	64.60	VRTA
L43	*L. casei*	81.97	90.81	VRTKAS
L47	*L. brevis*	75.17	75.87	RTKAS

amino acid metabolism and bacteriocin production. The discovery of plasmid DNA in the lactic acid bacteria is generally attributed to Cords *et al*. (1974) and has since been correlated with a number of commercially associated phenotypes in lactic acid bacteria, including lactose metabolism, proteinase activity, citrate fermentation, bacteriocin production, drug resistance, sugar transport and metabolism and the phage resistance mechanisms of restriction/ modification, adsorption resistance and abortive infection so on (Carr *et al.*, 2002; Cebra, 1999; Fernandes *et al*., 1987; Zhou *et al.*, 2000a). However, a key requirement for probiotic strains is that they should not carry any transmissible antibiotic resistance genes. Ingestion of bacteria carrying such gene is undesirable as horizontal gene transfer to recipient bacteria in the gut could lead to the development of new antibiotic resistant pathogen (Yamamoto and Takano, 1996; Nicas *et al.*, 1989; Olukoya *et al*., 1993). Considering this, in this study, the plasmid profiling and curing followed with antibiotic susceptibility test were carried out. All the seven strains lost their penicillin resistance after curing ensured that plasmid encode for resistance character and similar observations also reported by many researchers for different organisms (Caro *et al*., 1984; Carr *et al.*, 2002; Cebeci and Gürakan, 2003; Cohen, 1993; Cords *et al.*, 1974). However, the vanomycin resistance is not lost upon curing which indicates that such resistance is usually intrinsic it means chromosomally encoded and non transmissible. This is in accordance with earlier findings (Kanatani and Oshimura, 1994; Klein *et al.*, 1998; Ljungh and Wadstrom, 2006; Marcelo *et al*., 1998; Woodford *et al*., 1995).

Further, the loss of resistance after curing indicates the presence of the resistance character in plasmid and such strains were rejected (Table 4) considering the safety of the organisms. The strains of *Lactobacilli* and *Bifidobacteria* have long history of safe use in microbial adjunct nutrition (Zhou *et al*., 2000a). Therefore, the characterization of these bacteria, particularly in regard to antimicrobial resistance, is often neglected and this practice could become a problem, considering the strong expansion of the probiotic market as well as the increasing microbial drug resistance (Tynkkynen *et al*., 1998; Klein *et al*., 1998, Ljungh and Wadstrom, 2006). In fact, since probiotic bacteria are added to different kinds of products, they represent a potential source for the spread of antibiotic resistance genes (Perreten *et al*., 1997). Considering this fact, apart from plasmid curing studies, the other needed probiotic characters (antibiotic susceptibility, exopolysaccharide production and cholesterol reduction) were also carried out and the results are very much appreciable and comparable with earlier findings for existing probiotics (Zhou *et al*., 2000a, b). Over all, considering the results, the present study showed that, the selected lactic acid bacteria which have been isolated from our fermented milk sample can be a promising probiotics in future food and diary markets in our region.

CONCLUSION

From the present study we have selected 5 isolates which were not containing antibiotic resistance character in its plasmid to avoid spread of drug resistance. These selected organisms were considered for further screening process to fulfill the need of a probiotic in the food industry. All the isolates were characterized to the species level and was identified to be L10: *L. jungurthi*, L16: *L. reuteri*, L29: *L. plantarum*, L43: *L. brevis*, and L47. *L. casei*.

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REFERENCES

Acar, J.F. and F.W. Goldstein, 1991. Disk Susceptibility Test. In: Lorian, V. (Eds.), Antibiotics in Laboratory Medicine. 3rd Edn., Williams & Wilkins, New York.

Adams, M.R. and P. Marteau, 1995. On the safety of lactic acid bacteria from food. Int. J. Food Microbiol., 27: 263-264.

Ahn, C., D.C. Thompson, C. Duncan and M.E. Stiles, 1992. Mobilization and location of the genetic determinant of chloramphenicol resistance from *Lactobacillus plantarum* ca TC2R. Plasmid, 27: 169-176.

Anderson, D.G. and L.L. McKay, 1983. Simple and rapid method for isolating large plasmid DNA from lactic streptococci. Appl. Environ. Microbiol., 46: 549-552.

Arunachalam, K., H.S. Gill and R.K. Chandra, 2000. Enhancement of natural immune function by dietary consumption of *Bifidobacterium lactis* (HN019). Eur. J. Clin. Nutr., 54: 263-267.

Bauer, A.W., W.M.M. Kirby, J.C. Sherris and M. Turk, 1966. Antibiotic susceptibility testing by a standardized single disk method. Am. J. Clin. Pathol., 45: 493-496.

Billman-Jacobe, H., 1996. Expression in bacteria other than *Escherichia coli*. Curr. Opin. Biotechnol., 7: 500-504.

Caro, L., G. Churchward and M. Chandler, 1984. Study of plasmid replication *in vivo*. Meth. Microbiol., 17: 97-122.

Carr, F.J., D. Hill and N. Maida, 2002. The lactic acid bacteria: A literature survey. Critical Rev. Microbiol, 28: 281-370.

Cebeci, A. and C. Gürakan, 2003. Properties of potential probiotic *Lactobacillus plantarum* strains. Food Microbiol., 20: 511-518.

Cebra, J.J., 1999. Influences of microbiota on intestinal immune system development. Am. J. Clin. Nutr., 69: 46-51.

Cohen, S.N., 1993. Bacterial plasmids: Their extraordinary contribution to molecular genetics. Gene, 135: 67-76.

Cords, B.R., L.L. McKay and P. Guerry, 1974. Extrachromosomal elements in group N *Streptpcocci*. J. Bacteriolol., 117: 1149-1152.

Dubois, M., K.A. Gilles, J.K. Hamilton, P.A. Reberts and F. Smith, 1956. Colorimetric method for determination of sugars and related substances. Anal. Chem., 28: 350-356.

Fernandes, C.F., K.M. Shahani and M.A. Amer, 1987. Therapeutic role of dietary *Lactobacill*i and Lactobacillic fermented dairy products. FEMS Microbiol. Rev., 46: 343-356.

Frere, J., 1994. Simple method for extracting plasmid DNA from lactic acid bacteria. Lett. Appl. Microbiol., 18: 227-229.

Fuller, R., 1989. Probiotics in man and animals. J. Appl. Bacteriol., 66: 365- 378.

Ghosh, S., N.R. Mahapatra, T. Ramamurthy and P.C. Banerjee, 2000. Plasmid curing from an acidophilic bacterium of the genus *Acidocella*. FEMS Microbiol. Lett., 183: 271-274.

Gill, H.S., K.J. Rutherfurd and M.L. Cross, 2001b. Dietary probiotic supplementation enhances natural killer cell activity in the elderly: An investigation of age-related immunological changes. J. Clin. Immunol., 21: 264-271.

Grahn, E., S.E. Holm, H. Lilja and K. Sellgren, 1994. Interference of a Lactococcus lactis strain on the human gut flora and its capacity to pass the stomach and intestine. Scand. J. Nutr., 38: 2-4.

Ishiwa, H. and S. Iwata, 1980. Drug resistance plasmids in *Lactobacillus fermetum*. J. Gen. Appl. Microbiol., 26: 71-74.

Kalavathy, R., N. Abdullah, S. Jalaludin and Y.W. Ho, 2003. Effects of Lactobacillus cultures on growth performance, abdominal fat deposition, serum lipids and weight of organs of broiler chickens. Bri. Poult. Sci., 44: 139-144.

Kanatani, K. and M. Oshimura, 1994. Plasmid-associated bacteriocin production by a *Lactobacillus plantarum* strain. Biosci. Biotechnol. Biochem., 58: 2084-2086.

Klein, G., A. Pack, C. Bonaparte and G. Reuter, 1998. Taxonomy and physiology of probiotic lactic acid bacteria. Int. J. Food Microbiol., 41: 103-125.

Koneman, E.W., 1997. Antimicrobial Susceptibility Testing. In. Koneman, E.W., S.D. Allen, W.M. Janda, P.C. Schrecjenberger and W.C. Winn, (Eds.), Color Atlas and Textbook of Diagnostic Microbiology, 8th Edn., Lippincott, New York.

Kullen, M.J. and T.R. Klaenhammer, 1999. Genetic Modification of Intestinal Lactobacilli and Bifidobacteria. In. Tannock, G. (Ed.), Probiotics: A Critical Review, Horizon Scientific Press, Wymondham, U.K.

Lin, C.F., Z.F. Fung, C.L. Wu and T.C. Chung, 1996. Molecular characterization of a plasmid-borne (pTC82) chloramphenicol resistance determinant (cat-TC) from *Lactobacillus reuteri* G4. Plasmid, 36: 116-124.

Ljungh, A. and T. Wadstrom, 2006. Lactic acid bacteria as probiotics. Curr. Issues Intest. Microbiol., 7: 73-89.

Marcelo, G., E. Tolmasky, L.A. Actis and J.H. Crosa, 1998. Plasmids - A Practical Approach. In: Hardy, K.G. (Ed.), IRL Press, Oxford University Press, pp: 252-258.

Nicas, T.I., C.T. Cole, D.A. Preston, A.A. Schabel and R. Nagarajan, 1989. Activity of glycopeptides against vancomycin-resistant Gram-positive bacteria. Antimicrob. Agents Ch., 3: 1477-1481.

O'Sullivan, D.J. and T.R. Klaenhammer, 1993. Rapid mini-prep isolation of high-quality plasmid DNA from *Lactococcus* and *Lactobacillus* spp. Appl. Environ. Microbiol., 59: 2730-2733.

Olukoya, D.K., S.I. Ebigwi, O.O. Adebawo and F.O. Osiyemi, 1993. Plasmid profiles and antibiotic susceptibility patterns of Lactobacillus isolated from fermented foods in Nigeria. Food Microbiol., 10: 279-285.

Perdigon, G., S. Alvarez, M.E. Nader de Macias, M.E. Roux and A.P. de Ruiz Holgado, 1990. The oral administration of lactic acid bacteria increases the mucosal intestinal immunity response to enteropathogens. J. Food Prot., 53: 404-410.

Perreten, V., F. Schwarz, L. Cresta, M. Boeglin, G. Dasen and M. Teuber, 1997. Antibiotic resistance spread in food. Nature, 389: 801-802.

Posno, M., R.J. Leer, N. Van Luijk, M.J.F. Van Giezen, B.C. Lokman and P.H. Pouwels, 1991. Incompatibility of *Lactobacillus* vectors with replicons derived from small cryptic *Lactobacillus* plasmids and segregational instability of the introduced vectors. Appl. Environ. Microbiol., 57: 1822-1828.

Pouwels, P.H. and R.J. Leer, 1993. Genetics of *Lactobacilli*: Plasmids and gene expression. Antonie van Leeuwenhoek, 64: 85-107.

Rohlf, F.J., 1994. NTSYS-PC: Numerical taxonomy and multivariate analysis system version 2.2. State University of New York, Stony Brook, New York.

Rudel, L.L. and M.D. Morris, 1973. Determination of cholesterol using phthalaldehyde. J. Lipid Res., 14: 364.366.

Saarela, M., G. Mogensen, R. Fonden, J. Matto and T. Mattila-Sandholm, 2000. Probiotic bacteria: safety, functional and technological properties. J. Biotechnol., 84: 197-215.

Tynkkynen, S., K.V. Singh and P. Varmanen, 1998. Vancomycin resistance factor of Lactobacillus rhamnosus GG in relation toenterococcal vancomycin resistance (van) genes. Int. J. Food Microbiol., 41: 195-204.

Ulrich, S. and K.L. Friedrich, 1987. Identification of *Lactobacilli* from meat and meat products. Food Microbiol., 35: 1-27.

Vijayendra, S.V.N., G. Palanivel, S. Mahadevamma and R.N. Tharanathan, 2008. Physico-chemical characterization of an exopolysaccharide produced by a non-ropy strain of *Leuconostoc* sp. CFR 2181 isolated from dahi, an Indian traditional lactic fermented milk product. Carbohyd. Polym., 72: 300-307.

Woodford, N., A.P. Johnson, D. Morrison and D.C.E. Speller, 1995. Current perspective on glycopeptide resistance. Clin. Microbiol. Rev., 8: 585-615.

Yamamoto, N. and T. Takano, 1996. Isolation and characterization of a plasmid from *Lactobacillus helveticus* CP53. Biosci. Biotechnol. Biochem., 60: 2069-2070.

Zhou, J.S., P.K. Gopal and H.S. Gill, 2000a. Potential probiotic lactic acid bacteria *Lactobacillus rhamnosus (*HN001), *Lactobacillus acidophilus* (HN017) and *Bifidobacterium lactis* (HN019) do not degrade gastric mucin *in vitro*. Int. J. Food Microbiol., 63: 81-89.

Zhou, J.S., Q. Shu, K.J. Rutherfurd, J. Prasad, M.J. Birtles, P.K. Gopal and H.S. Gill, 2000b. Safety assessment of potential probiotic lactic acid bacterial strains *Lactobacillus rhamnosus* HN001, Lb. acidophilus HN017, and *Bifidobacterium lactis* HN019 in BALB/c mice. Int. J. Food Microbiol., 56: 87-96.