Journal of Microbiology and Antimicrobials Vol. 4(1), pp. 16-22, January 2012
Available online http://www.academicjournals.org/JMA
DOI: 10.5897/JMA11.083
ISSN 2141-2308 ©2012 Academic Journals

Full Length Research Paper

Isolation of *Lactobacillus plantarum* from cow milk and screening for the presence of sugar alcohol producing gene

A. Mohan Kumar [1]* and N. Murugalatha[2]

[1]Department of Zoology, Chikkanna Government Arts College, Tirupur-638602, Tamilnadu, India.
[2]Department of Microbiology, Hindusthan College of Arts and Science, Coimbatore- 614 028, Tamilnadu, India.

Accepted 30 July, 2011

***Lactobacillus plantarum*, was isolated from cow milk, characterized by partial 16S rRNA gene sequencing and phenotypic features and resoluted for the presence of bacteriocin and their activity against pathogenic organism. The organism showed very strong activity against *Streptococcus faecalis* and strong activity against *Bacillus mycoides, Klebsiella pnuemoniae*, *Staphylococcus aureus* and *Proteus vulgaris*. The organisms *Psuedomonas aeruginosa* and *Salmonella typhi* were resistant to the isolate. For the isolate, low calorie sugar alcohol, namely sorbitol, producing genes were screened and sequenced.**

Key words: *Lactobacillus plantarum,* antimicrobial activity, sorbitol producing gene.

INTRODUCTION

Lactobacillus plantarum, a probiotic organism plays a major role in making the intestine a home for beneficial organism and a barrier for pathogens. It is largely found as the dominant species in the last step of natural food raw- material fermentation, including a variety of vegetables, meat and milk (Daeschel et al., 1987; Hammes et al., 1990). Lactic acid is produced from its sugar metabolism.

Lactic acid bacteria are a group of microorganism widely used in the industrial food fermentation. They are the cell factors for the production of high-value metabolites involved in flavor, texture or health (De Vos et al., 2004)

Sorbitol, also known as glucitol, is a sugar alcohol used as a sugar substitute. It is referred to as a nutritive sweetener as it provides dietary energy which is used in diet foods, mints, cough syrups etc., Sorbitol is a low calorie non metabolized sugar alcohol that can replace sucrose or lactose in food products, with nearly equivalent sweetness and taste (Salminen et al., 1993). These compounds have a stabilizing effect on food by partially mimicking fat (Debord et al., 1987). The development of novel dairy products naturally enriched in polyols during fermentation processes offers interesting perspectives (Hoefnagel et al., 2002).

In our present study *L. plantarum* was isolated from cow milk samples and was screened for the presence of sorbitol producing gene.

*Corresponding author. E-mail: moniver@satyam.net.in.

MATERIALS AND METHODS

Isolation of lactic acid bacteria

Raw unpasteurized milk samples of cow were collected from the local area of Coimbatore during lactation period under aseptic conditions in a sterile screw cap tubes, processed within three hours and used for further studies.

Milk samples were serially diluted in peptone medium and incubated at 23ºC for 30 min before plating by which 50% of recovery of Lactic acid bacteria (LAB) was increased. Diluted samples were plated onto De Man Rogosa Sharpe (MRS) medium for *Lactobacillus* isolation and incubated at 37ºC for 48-72 h. Well-isolated colonies with typical characteristics namely pure white, small (2 - 3 mm diameter) with entire margins were picked from each plate and transferred to MRS broth.

Identification of* lactic acid bacteria *(LAB)

Identification of the *Lactobacilli* was performed according to their morphological, cultural, physiological and biochemical characteristics (Kandler and Weiss, 1986; Sharpe et al., 1979): Gram reaction, production of catalase, carbohydrate fermentation patterns, growth at 15 and 45°C in the *lactobacilli* de Man Rogosa and Sharpe (MRS) broth as described by Bergey's Manual of systematic Bacteriology, methyl red and Voges-Proskauer test in MRVP medium, nitrate reduction in nitrate broth, and indole production in Tryptone broth. Purified cultures were maintained at -20°C in MRS broth with 10% glycerol and enriched in MRS broth by incubating at 37°C for 24 h.

Agar well diffusion assay

The isolated strains were grown in MRS broth at 37ºC for 48 h. Cells were separated by centrifugation at 5000 rpm for 10 min. Around 6 mm diameter wells were made on pre inoculated agar media and each well was filled with 100 µl of culture supernatant of *L. plantarum* after neutralization with NaOH. Inhibitory activity was performed against certain Gram positive and Gram negative organisms like *Lactobacillus acidophilus* (MTCC447), *Lactobacillus delbrueckii* subsp. *lactis* (MTCC 911), *Lactococcus lactis* subsp *lactis* (MTCC 440), *Bacillus amyloliquifaciens* (MTCC 1270), *Bacillus cereus* (MTCC 1272), *Bacillus mycoides* (MTCC 645), *Klebsiella pneumoniae* (MTCC 3384), *Staphylococcus aureus* (MTCC 740), *Streptococcus faecalis* (MTCC 459), *Pseudomonas aeruginosa* (MTCC 647), *Proteus vulgaris* (MTCC 744), and *Salmonella typhi* (MTCC 531). Inhibition zones around the wells were measured and recorded (Toba et al., 1991).

Sweetening gene isolation from bacteriocin producing *Lactobacillus* isolates

16s rRNA sequencing of sorbitol producing Lactobacillus plantarum

DNA Isolation: 1.5 ml of broth was taken in a microfuge tube and centrifuged at 5,000 rpm for 15 min. The supernatant was discarded. To the pellet, 1.5 ml of broth was added and again centrifuged at 8,000 rpm for 10 min. 467 µl of TE buffer, 50 µl of 0.5% lysozyme and 30 µl of 10% SDS was added. Then 3 µl of proteinase k was added (20 mg/ml). It was mixed well and incubated for 1 h at 37°C. After the incubation, equal volume of phenol: chloroform (25:24) mixture was added and mixed well by inverting the tubes gently until the phases are completely mixed. Then it was centrifuged at 8,000 rpm for 10 min. After centrifugation, the upper aqueous phase was transferred to a new tube and 1/10 volume of sodium acetate was added. Then 0.6 volume of isopropanol was added and mixed gently until the DNA gets precipitated and then centrifuged at 8,000 rpm for 10 min. To the pellet, 70% ethanol was added and centrifuged at 5,000 rpm for 10 min. To the pellet, 20 µl of TE buffer was added and stored in ice cold condition (Sambrook et al., 1989) and analyzed by Agarose gel electrophoresis.

PCR amplification of 16s rRNA gene: The polymerase chain reaction (PCR) was performed in 0.2 ml micro centrifuge tube by adding 2 µl of template DNA, 3 µl of forward primer-5' GTTCGCCACTCACTCAAATG-3', 3 µl of reverse primer-5'GGCGTGCCTAATACATGCAA-3', 20 µl of PCR master mix and 12 µl of nuclease free water for 40 µl reactions and homogenized by quick spin. The reaction was performed with initial heating at 94°C for 5 min, 35 cycles of denaturation at 94°C for 30 s, annealing at 50°C for 1.5 min, extension at 72°C for 30 s, followed by final extension at 72°C for 10 min. The reaction tubes were cooled and a small aliquot of PCR product was run on agarose gel electrophoresis along with the DNA molecular weight marker to analyse the product.

Elution of product from the gel

The DNA fragment from the agarose gel was cut with a clean, sharp cutter. The gel slice was weighed using weigh balance and put in a 2 ml micro centrifuge tube. To one volume of gel slice 3 volumes of gel extraction buffer was added (100 mg-100 µl). The tubes were incubated at 55°C for 5-10 min until the gel piece has completely dissolved. The sample was mixed well by inverting the tube every 2-3 min during the incubation to solubilize the agarose completely. After the gel piece has dissolved completely, one gel volume of isopropanol was added to the tube and mixed well (e.g. for 100 mg gel piece add 100 µl isopropanol). The spin column was placed in a collection tube provided. The gel extracted solution with isopropanol was loaded onto the spin column (600 µl each time). Then the mixture was spin at 13,000 rpm for 1 min at room temperature. After centrifugation, the content of the collection tube was discarded and place the spin column back in the same collection tube. To the column, 500 µl of wash buffer was added and the mixture was centrifuged at 13,000 rpm for 1 min at room temperature. The contents of the collection tubes were discarded and the spin column was kept back in the same collection tube. This step was repeated once again. The empty column was spin along with the collection tube at 13,000 rpm for 3 min at room temperature. The spin column was placed in a fresh 1.5 ml micro centrifuge tube. To the spin column, 20 µl of elution buffer was added and the vial along with the spin column was kept at room temperature for 2 min. Then, it was centrifuged at 13,000 rpm for 1 min at room temperature. 20 µl of elution buffer was added again to the spin column and kept for 2 min at room temperature. Then, it was centrifuged at 13,000 rpm for 1 min at room temperature and was subjected to sequencing.

Sequencing and phylogenetic analysis

The PCR product was sequenced bi-directionally using the forward, reverse and internal primer. Sequences of the fragments were determined by the automatic Big Dye (dideoxy chain terminator) sequencer ABI 3130 Genetic Analyzer. Taxonomic strain identification was performed by comparing the sequences of each isolate with those reported in the Basic BLAST database (Altschul et al., 1997). A distance matrix and phylogenetic tree was generated using the neighbor-joining method using GENETYX software (Genetyx Corporation).

Screening of sorbitol producing gene from Lactobacillus plantarum

The two sorbitol producing genes sorbitol-6-phosphate dehydrogenase srlD1 and srlD2 identified in the genome sequence were amplified by PCR (Sambrook et al., 1989) using primers (5'-CCGCAACGTTAATCGTAACC-3') and (5'-CGGGGCGATTGTTTATAATG-3') for srlD1 and (5'-TTGATGCATAATGATTAATATTTCG-3') and (5′-TTGTCTAGACATTGCCTCACCATGC-3′) for srlD2. The PCR reaction was performed in 0.2 ml micro centrifuge tube by adding 4 µl of template DNA, 2 µl of forward primer, 2 µl of reverse primer, 25 µl of PCR master mix and 17 µl of nuclease free water for 50 µl reactions and homogenized by quick spin. The reaction was performed with initial heating at 94°C for 5 min, 35 cycles of denaturation at 94°C for one min, annealing at 55°C for 1 min, extension at 72°C for 1 min followed by final extension at 72°C for

Table 1. Biochemical characterization of Lactobacillus isolated from cow milk.

Milk sample	Gram reaction	Motility	Growth		Indole	MR	VP	Citrate	Catalase	Nitrate reduction	Gelatin
			15ºC	45ºC							
Cow	Rod	-	+	-	-	-	-	-	-	-	-

Table 2. Phenotypic profile of *Lactobacillus* from cow milk.

Isolates	Arabinose	Cellobiose	Lactose	Mannitol	Melibiose	Salicin	Sorbitol	Sucrose	Raffinose	Trehalose
L. plantarum	-	+	+	+	+	+	+	+	+	+

10 min. The reaction tubes were cooled and a small aliquot of PCR product was run on agarose gel by electrophoresis along with the DNA molecular weight marker (250 bp-10Kb) to analyse the product. The gel was eluted and subjected to sequencing.

RESULT

Identification of LAB

The strain reacted positively to gram staining under a light microscope. *Lactobacilli* are generally long rods. *Lactobacillus* do not possess flagella and do not create endospores, nitrates are not reduced, gelatin is not liquefied, indole is not produced, acidic and non acidic end products are not produced and are catalase negative (Table 1).

The identified genus *Lactobacillus* was further classified to the species level. Strains were able to ferment sugars at different percentages which were much significant for identification of the species. *L. plantarum* was able to ferment all the sugars except for Arabinose (Table 2).

Agar well diffusion assay

The culture supernatant obtained *from L. plantarum* was tested for antibacterial activity against both Gram positive and Gram-negative bacteria. *B. mycoides*, *K. pneumoniae*, *S. faecalis, S. aureus* and *P. vulgaris* were among the sensitive bacteria tested. It showed very strong inhibition against *S. faecalis* with the zone of inhibition of 16 mm in diameter and against *P. vulgaris* with the zone of inhibition of 15mm in diameter. *B. mycoides*, *S. aureus* and *P. vulgaris* were strongly inhibited with zone of inhibition 10-14 mm in diameter.

Sweetening gene isolation from bacteriocin producing Lactobacillus isolates

16s rRNA sequencing of sorbitol producing L. plantarum

Bacterial DNA was extracted by phenol-chloroform method, amplified using PCR with specific *L. plantarum* primers of 16s F : 5' GTTCGCCACTCACTCAAATG-3'; 16s R: 5'GGCGTGCCTAATACATGCAA-3'and processed by agarose gel electrophoresis. Fragment with 409 bp were excised (Plate 1), re-amplified, purified and the region of 16s rRNA gene was analysed with ABI 3130 BigDye Terminator Genetic Analyzer (Figure 1). Phylogenetic tree was constructed with the sequences of the PCR product and was compared with known gene sequences in GenBank by multiple-sequence alignment using GENETIX software (Figure 2). Phylogenetic analysis showed that the isolate CWL6 was found to be most similar to *L. plantarum* strain IMAU70089 (NCBI GenBank Acc.No: GQ131205.1) and a maximum of 94% identity was reputable. 84% of maximum identity was found with that of *Lactobacillus rogosae* strain ATCC 27753 (NCBI GenBank Acc No: GU269544.1) and 87% maximum similarity with Lactobacillus sp. oral taxon A89 clone (NCBI GenBank Acc No. GU428971.1), indicating that the isolate was as strain of *L. plantarum* (NCBI GenBank Acc. No: HM448901.1). Information about other close homologue for the microbe can be found from the alignment view table (Table 3).

Screening of sorbitol producing gene from *Lactobacillus plantarum*

Sorbitol is a low-calorie sugar alcohol used as an ingredient in the food industry based on its sweetness and high solubility. The two sorbitol-6-phosphate dehydrogenase (stl6PDH) genes srlD1 and srlD2 were screened for their presence in *L. plantarum* by PCR amplification using the appropriate primers. srlD1 gene (NCBI GenBank Acc No. HQ395383.1) was identified from the isolated strain and was sequenced, where as srlD2 gene was not identified (Figure 3 and Plate 2).

DISCUSSION

Milk samples from cow were collected from local area of

>ML 16Sr-01

CAAATGACCACGTTTTTTCCCCATCCTGGCGGACGGGTGAGTAACGCGTAGGAATCTATCCGTGGGTGGGGGATAACTCTGGGAA

ACTGGAGCTAATACCGCATGATACCTGAGGGTCAAAGGCGCAAGTCGCCTGCGGAGGAGCCTGCGTTTGATTAGCTTGTTGGTGG

GGTAATGGCCTACCAAGGCGATGATCAATAGCTGGTCTGAGAGGATGATCAGCCACACTGGGACTGAGACACGGCCCAGACTCCT

ACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGGGCAACCCTGATCCCCAATGCCCGTGTGTGAATAAGTTTTCCTATTGTAC

AGTTTTCCGACATGATACAGGCCGTAATCTACACATATCTGCATCGAGAGGCCACTTCAGACCAGTACG

Figure 1. Aligned Sequence Data: (409 bp) of *Lactobacillus plantarum.*

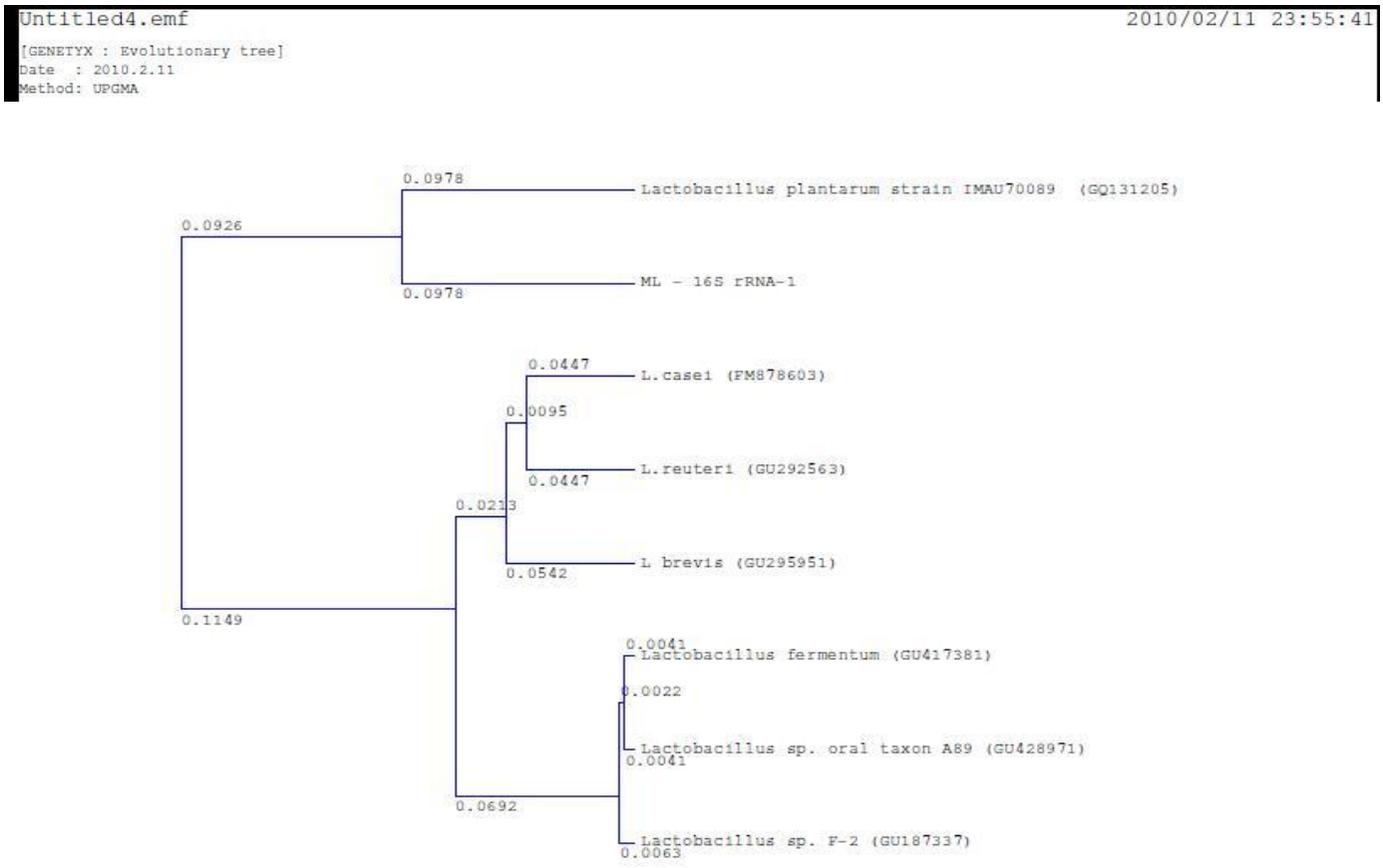

Figure 2. Phylogenetic tree

coimbatore and processed for isolation of LAB. The microbial colonies were counted in raw milk samples by standard plate count. The colonies in raw milk sample are expected a little higher than real microflora. This is due to contamination from the animal, especially the exterior of the udder and the adjacent areas; bacteria found in manure, soil and water may enter (Garbutt, 1997).

Leuconostoc and *Lactobacillus* both were found higher in number in camel milk as compared to cow, buffalo and goat milk. But the total number of bacteria was found higher in cow milk (Singh and Rakesh, 2009).

The LAB isolates were classified into the genera *Lactobacillus* based on their morphological and biochemical characters (Sharpe et al., 1979). The

Table 3. Alignment view and distance matrix table (With Culture No. 1 sequence taken as reference sequence) Sequences producing significant alignments:.

Accession	Description	Query coverage (%)	E value	Max identity (%)
GQ131205.1	*Lactobacillus plantarum* strain IMAU70089 16S ribosomal RNA gene, partial sequence	76	1e-134	94
GU269544.1	*Lactobacillus rogosae* strain ATCC 27753 16S ribosomal RNA gene, partial sequence	48	4e-50	84
GU428971.1	*Lactobacillus* sp. oral taxon A89 clone ED016 16S ribosomal RNA gene, partial sequence	40	2e-47	87
GU428970.1	*Lactobacillus* sp. oral taxon A89 clone ED015 16S ribosomal RNA gene, partial sequence	40	2e-47	87
GU428969.1	Lactobacillus sp. oral taxon A89 clone ED014 16S ribosomal RNA gene, partial	40	2e-47	87
GU417381.1	*La fermentum* clone RW005 16S ribosomal RNA gene, partial sequence	40	2e-47	87
GU417380.1	*Lactobacillus fermentum* clone RV052 16S ribosomal RNA gene, partial sequence	40	2e-47	87
GU417378.1	*L. fermentum* clone IM053 16S ribosomal RNA gene, partial sequence	40	2e-47	87
GU417377.1	*L. fermentum* clone IM046 16S ribosomal RNA gene, partial sequence	40	2e-47	87
GU417376.1	*L. fermentum* clone IM042 16S ribosomal RNA gene, partial sequence	40	2e-47	87
GU417368.1	*L. fermentum* clone ED008 16S ribosomal RNA gene, partial sequence	40	2e-47	87
GU417341.1	*L. fermentum* clone ATC_H36_19 16S ribosomal RNA gene, partial sequence	40	2e-47	87
GU417339.1	*L. fermentum* clone RV025 16S ribosomal RNA gene, partial sequence	40	2e-47	87
GU417338.1	*L. fermentum* clone IN020 16S ribosomal RNA gene, partial sequence	40	2e-47	87
GU417337.1	*L. fermentum* clone ED005 16S ribosomal RNA gene, partial sequence	40	2e-47	87
GU299088.1	*L. fermentum* strain O-1 16S ribosomal RNA gene, partial sequence	40	2e-47	87
GU299086.1	*L. fermentum* strain 3-2 16S ribosomal RNA gene, partial sequence	40	2e-47	87
GU187337.1	*Lactobacillus* sp. F-2 16S ribosomal RNA gene, partial sequence	40	2e-47	87
GU177632.1	*L. fermentum* strain G-10 16S ribosomal RNA gene, partial sequence	40	2e-47	87
GQ141810.1	*L. fermentum* strain Kx293A3 16S ribosomal RNA gene, partial sequence	40	2e-47	87
EU825661.1	*L. fermentum* strain 3 16S ribosomal RNA gene, partial sequence	40	2e-47	87
EU825658.1	*L. fermentum* strain 1 16S ribosomal RNA gene, partial sequence	40	2e-47	87
GQ131282.1	*L. fermentum* strain IMAU70167 16S ribosomal RNA gene, partial sequence	40	2e-47	87
GQ131281.1	*L. fermentum* strain IMAU70166 16S ribosomal RNA gene, partial sequence	40	2e-47	87
GQ131280.1	*L. fermentum* strain IMAU70165 16S ribosomal RNA gene, partial sequence	40	2e-47	87
GQ131278.1	*L. fermentum* strain IMAU70163 16S ribosomal RNA gene, partial sequence	40	2e-47	87
GQ131274.1	*L. fermentum* strain IMAU70159 16S ribosomal RNA gene, partial sequence	40	2e-47	87
GQ131273.1	*L. fermentum* strain IMAU70158 16S ribosomal RNA gene, partial sequence	40	2e-47	87
GQ131272.1	*L. fermentum* strain IMAU70157 16S ribosomal RNA gene, partial sequence	40	2e-47	87
GQ131271.1	*L. fermentum* strain IMAU70156 16S ribosomal RNA gene, partial sequence	40	2e-47	87
GQ131270.1	*L. fermentum* strain IMAU70155 16S ribosomal RNA gene, partial sequence	40	2e-47	87
GQ131268.1	*L. fermentum* strain IMAU70153 16S ribosomal RNA gene, partial sequence	40	2e-47	87
GQ131267.1	*L. fermentum* strain IMAU70152 16S ribosomal RNA gene, partial sequence	40	2e-47	87
GQ131266.1	*L. fermentum* strain IMAU70151 16S ribosomal RNA gene, partial sequence	40	2e-47	87
GQ131265.1	*L. fermentum* strain IMAU70150 16S ribosomal RNA gene, partial sequence	40	2e-47	87
GQ131264.1	*L. fermentum* strain IMAU70149 16S ribosomal RNA gene, partial sequence	40	2e-47	87

<srlD1> partial seq. 1

CCGCAACGTTAATCGTAACCCCGGTAATGTAACTGGCACGTTCTGATAGTAAATAGTTAACTGCATCCGCAACTTCTGATA

GTTTGCCACTCCGACCAAGAGGTGTGGTTGACGTACTCTTATAGCCTGCCCGAATGTCATCAACAGTTTTATGCCGGGTAT

TAGCTAACGCCTCTTCATAAGAGCGTGTTCTTAGCCCAGTAGCTTCCATAAGACCTGGCGCGACACCAACAATTCGAATAT

TATGTTCACCTAATTCTTTAGCCCACGAACGAGTGAACCCATTAATCGCTCCCTTAGTTGCTGAATAAACACTCTGTCCTTG

TGAACCTTCAAGTCCAGCTTCTGAGGACATATTAACAATAACACCCTACTGTTGCTTCTCGAATTGATGGGTAGCTGCCTG

CGATACAAGGAAGACGCTTTTCACGTTCACGGCAAACATTTTCACAAAATCCGATTCTGCAAATTCATACTTACCGTGAGG

ATCCTTGGCGTCAGCCAATAAACGTGGCAAATTGATACCAGCATTATTTACAAGCCCGTCAATTTTTCCTTGTTCATCAACA

ACCTTTAAGACCAGCCGATTAACCGCTTCCGCATCTGTGA

Figure 3. *srl*D1 sweetening gene partial sequence.

predominant *Lactobacillus* sp. was further classified to the species level (Kandler and Weiss, 1986). The differentiating characteristics of *L. plantarum* are given in Table 1. *L. plantarum* were able to grow at 15°C. De Man et al. (1960) stated that Lactobacilli are generally isolated on rich media such as MRS, which routinely used for the isolation and counting of *Lactobacilli* for most fermented food products. *Lactobacillus* isolates from temperate regions were classified according to their morphology, physiology and molecular characteristics in which few strains were able to utilize citrate and were found to be non motile, catalase, indole, VP negative, nitrates are not reduced and gelatin was not liquefied (Kandler and Weiss, 1986). Sphelhaug et al. (1989) reported that MRS agar was suitable for *lactobacilli* bacteriocin assay.

The inhibitory spectrum of the cell free supernatant fluid against variety of Gram positive and negative pathogens was widely varied. It was observed that *L. plantarum* had an inhibitory effect on *S. faecalis*, *B. mycoides, S. aureus*, *K. pneumoniae* and *P. vulgaris*. However, none of them affected *Bacillus amyloliquefaciens*, *S. typhi*, *P. aueruginosa*, *Bacillis cereus*. Researchers have reported that naturally occurring antimicrobials produced by certain bacteria are effective at controlling undesirable microorganisms in cottage cheese (Weber and Broich 1986; Tortorello et al., 1991). An expanded host range has been noted recently for a number of *Lactobacillus* bacteriocins which kill *Enterococcus faecalis, Listeria monocytogenes*, *Clostridium botulinum*, *Candida tyrobutyricum*, *S. aureus* and *Aeromonas hydrophila* (Klaenhammer, 1993). Toba et al. (1991) determined bacteriocins in six *Lactobacillus gasseri*, *L. acidophilus* JCM 1132 and *L. acidophilus* LAPT 1060 strains from infant feces active against other *Lactobacillus* strains.

PCR analysis of *L. plantarum* was subjected to partial sequencing and the organism was confirmed to be *L. plantarum* and partial 16S rRNA gene sequence was deposited in NCBI, Gen Bank (HM448901). Especially the probiotic organisms like *Lactobacillus* have gained its importance in producing sweeteners at high level. It has been described by De Boeck et al. (2010) that a sorbitol-producing *Lactobacillus casei* (strain BL232) in which the *gut*F gene, encoding a sorbitol-6-phosphate dehydrogenase, was expressed from the lactose operon that accumulated sorbitol in the medium that was rapidly metabolized after glucose exhaustion. The two sorbitol-6-phosphate dehydrogenase (Stl6PDH) genes (*srl*D1 and *srl*D2) identified in the genome sequence were constitutively expressed at a high level for the production of sorbitol from fructose 6- phosphate in the mutant strain *L. plantarum (*Victor et al. 2007).

In conclusion the microbiota from milk is efficient in inhibiting the pathogenic organism, will act as a barrier by developing its antimicrobial activities in the host system of defense and the inhibitory spectrum of the antimicrobial substance has a potential application as a biopreservative in food industry. Spontaneous resistance of *lactobacilli* to a wide range of clinically important antibiotics may enable the development of antibiotic and probiotic combination therapies for several infections and for the development of infant probiotics products.

Development of novel functional food with the polyol-sorbitol on the grounds of industrial applications and the health promoting benefits under *in situ* production during the fermentation process or as an additive is of exceptional significance.

It would be of interest whether the *L. plantarum* bearing *srl*D1 gene alone can exhibit its activity to produce the low cholesterol sugar sorbitol which would be very much a natural product from milk when it is inhabited and can be used as an ingredient on its sweetness and its high solubility.

REFERENCES

Altschul SF, Madden TL, Schäffer AA, Zhang JH, Zhang Z, Miller W, Lipman DJ (1997). Gapped BLAST and PSI-BLAST: A new generation of protein database search programs. Nucl. Acids. Res., 25: 3389–3402.

Daeschel F, Torriani S (1987). Physiological characters and distribution of Lactic acid bacteria isolated from maize silage. J. Appl. Bacteriol., 60: 83-92.

Debord B, Lefebvre C, Guyothermann AM, Hubert J, Bouche R and Guyot JC (1987). Study of different crystalline forms of mannitol: comparative behavior under compression. Drug Dev. Ind. Pharm. 13:1533-1546.ic-acid bacteria in meat fermentation. FEMS Microbiol. Rev., 87: 165-173.

De Boeck R, Sarmiento-Rubiano LA, Nadal I, Monedero V, Perez-Martinez G, Yebra MJ (2010). Sorbitol production from lactose by engineered Lactobacillus casei deficient in sorbitol transport system and mannitol-1-phosphate dehydrogenase. Appl. Microbiol. Biotechnol., 85: 1915-1922.

De Man JC, Rogosa M, Sharpe ME (1960). A medium for the cultivation of lactobacilli. J. Appl. Bacteriol.,, 23: 130-1 35.

De Vos, Willem M, Hugenholtz, Jeroen (2004). Engineering metabolic highways in lactococci and other lactic acid bacteria. Trends in Biotechnol., 22(2): 72-79.

Garbutt (1997). Essentials of food Microbiology. Arnold International edition, pp. 67-72.

Hammes WP, Bantleon A, Min S (1990). Lactic acid bacteria in meat fermentation. FEMS Microbiol. Rev., 87: 165-174.

Hoefnagel MH, Starrenburg MJ, Martens DE, Hugenholtz J, Kleerebezem M, Van Swam II, Bongers R, Westerhoff HV, Snoep JL (2002). Metabolic engineering of lactic acid bacteria, the combined approach: kinetic modeling, metabolic control and experimental analysis. Microbiology, 148: 1003-1013.

Kandler O, Weiss N (1986). Genus Lactobacillus In: Bergey's Manual of Systematic Bacteriology, (Sheath PHA, ed). 2: 1209-1234.

Klaenhammer TR (1993). Genetics of bacteriocins produced by lactic acid bacteria. FEMS Microbiol. Rev., 12: 39-86.

Salminen S, Ramos P, Fonden R (1993). Substrates and lactic acid bacteria,. In Salminen S, von A Wright (ed.), Lactic acid bacteria. Marcel Dekker Inc., New York, NY. pp. 295-313.

Sharpe ME, Fryer TF, Smith DG (1979). Identification of Lactic acid Bacteria. In: Identification methods for Microbiologists, Gibbs EM, Skinner FA (Eds), London: Academic Press, pp. 233-259.

Sambrook J, Fritsch EF, Maniatis T (1989). Molecular Cloning: a Laboratory Manual, 2nd ed. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.

Singh GP, Rakesh Roshan Sharma (2009). Dominating species of Lactobacilli and Leuconostocs present among the lactic acid bacteria of milk of different cattle's. Asian J. Exp. Sci., 23(1): 173-179.

Sphelhaug SR, Hardlender SK (1989). Initiation of food born bacterial pathogens by bacteriocins from Lactococcus lactis and Pediococcus spentosaceous. J. Food. Prot., 52: 856-862.

Tortorello ML, Best S, Batt CA, Woolf HD, Bencher J (1991). Extending the shelf life of cottage cheese. Identification of spoilage flora and their control using food grade preservatives. Cult. Dairy Prod. J., 26: 8-9.

Toba T, Yoshioka E, Itoh T (1991). Acidophilucin A, a new heat – labile bacteriocin produced by Lactobacillus acidophilus LAPT 1060. Lett. Appl. Microbiol., 12: 106-108.

Weber GH, Broich WA (1986). Shelf life extension of cultured dairy foods. Cult. Dairy Prod. J., 21: 19-2.

Victor L, Ana R, Anne W, Philippe G, André S, Michiel K, Jeroen H, Eddy JS, Pascal H (2007). High-Level Production of the Low-Calorie Sugar Sorbitol by Lactobacillus plantarum through Metabolic Engineering. Appl. Environ. Microbiol., 73(6): 1864-1872.