Internet Journal of Food Safety, Vol.15, 2013, p.39-42


# Isolation of Lactic Acid Bacteria from Fermented Milk Products and Their Antimicrobial Activity against *Staphylococcus aureus*

Noopur Agrawal[1] and Alka Prakash[1]*

[1]*Environmental Biotechnology Lab, Department of Zoology, Dayalbagh educational Institute, Agra-282005, India.*

**Abstract**

*A total of 13 isolates of lactic acid bacteria (LAB) were isolated from curd and cheese samples. The samples were collected from the different regions of the Agra city. The isolates were identified as Lactococcus sps. (23%), Pediococcus sps. (54%), Lactobacillus sps. (15%) and Leuconostoc sps. (18%) by biochemical characterization. These isolates were tested for their antimicrobial activity against Standard S.aureus (MTCC 3381) by well diffusion method and MIC of all these isolates were observed to check the sensitivity of each isolate. Effect of LAB on the S.aureus was studied by SDS-PAGE.*

**Keywords: Lactic acid bacteria (LAB), Antimicrobial activity, MIC, Staphylococcus aureus.**

## Introduction

Lactic acid bacteria (LAB) are a group of related bacteria that produce lactic acid as a result of carbohydrate fermentation. They comprise a clade of Gram positive, usually non motile acid tolerant microorganisms. They are generally non-spore forming, non-respiring cocci, coccobacilli or rods. LAB growth lowers both the carbohydrate content of the food that they ferment and the pH due to the lactic acid production. Certain LAB strains have been reported to be highly antagonistic to biofilm forming *S.aureus* (Ammor *et al*, 2006).

LAB strains are potentially promising because they generate bactericidal bioactive peptides (bacteriocins) and enzymes that are able to control biofilm formation and growth of pathogens (Millette *et al*, 2006).

*Corresponding author mailing address: prakashdr.dei@gmail.com

LAB exerts strong antagonistic activity against many microorganisms, including food spoilage organism and pathogens. Some strains may contribute to food preservation of fermented food by producing bacteriocins (Brink *et al*, 1994). The major parameters involved in bacterial growth inhibition are the pH, which decreases by the production of organic acid, nutrient competition, hydrogen peroxide and antibiotic production. *S.aureus* is a Gram positive coccus, non-motile non-spore forming facultative anaerobic which appears as grape like clusters. It is a common pathogen associated with hospital acquired diseases which causes major problem for public health. One of the major causes of staphylococcal enterotoxin is vomiting and diarrhea when ingested and is responsible for staphylococcal food poisoning (Nostro *et al*, 2002).

## Materials and Methods

**Microorganism** The pathogen namely Staphylococcus aureus MTCC 3381 was procured from Institute of Microbial Technology, Chandigarh, India and LAB was isolated from samples of fermented milk products of curd and cheese collected from various regions of Agra city.

**Identification of Lactic acid bacteria** MRS Agar and MRS broth were used for enumeration and culture of Lactic acid bacteria at 37℃ for 24 hrs. The cultures were identified by performing various morphological and biochemical characterization. It includes Gram reaction, Spore formation, Glucose production, hot loop test and Sugar fermentation test. After confirmation of LAB isolates, turbidity were matched with the McFarland standard series. Out of 13 isolates, 6 isolates were having 15 × 108 CFU/ml and 7 isolates were having 12 × 108 CFU/ml.

**Determination of Antimicrobial Activity of LAB isolates against *S.aureus*** The antimicrobial activity of isolated LAB S.aureus was performed by well-diffusion method (Al-Allaf et al, 2009). S.aureus was incubated in BHI broth at 37° C at 24 hrs. 15ml of Muller Hinton Agar was prepared and 150µl of S.aureus culture having 6 × 108 CFU/ml was inoculated into it. Once solidified the plates, wells of 6mm diameter were made and 40µl of each concentration of LAB isolates were filled into well. Then the plates were incubated at 37℃ for 24 hrs. and antimicrobial activity was determined by measuring the clear zone around the well (Figure 1).

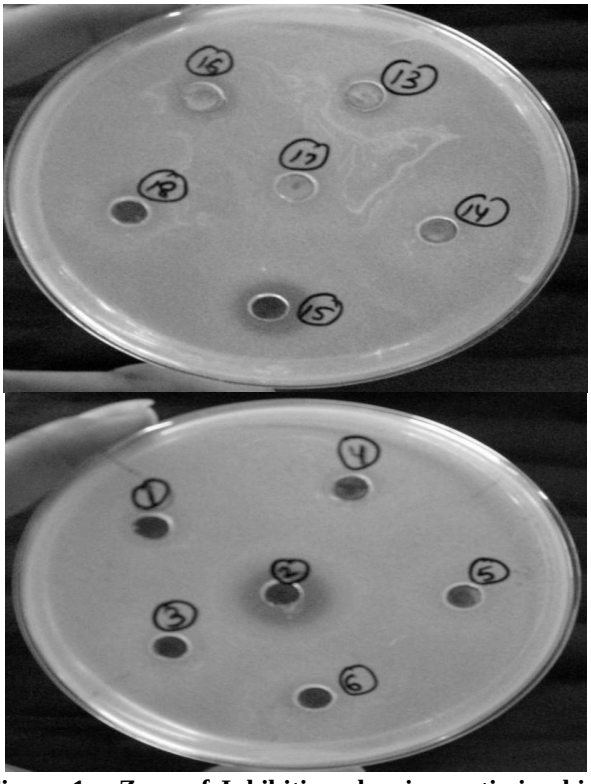


**Figure 1. Zone of Inhibition showing antimicrobial activity against S.aureus at diffent concentration of LAB isolates**

**Preparation of Protein Samples** Swabs were taken from inhibitory zones of 6 LAB isolates having 6 × 107 CFU/ml, 7 LAB isolates having 4.8 × 107 CFU/ml, 3 LAB isolates (S1, S2, S13) having 0.66×107 CFU/ml and 1 LAB isolate (S12) having 0.53×107 CFU/ml with the help of sterile loop each was inoculated in 3 ml of BHI broth and incubated at 37℃ for 24 hrs. Then 1.5 ml of sample was taken and was centrifuged for 7 minutes at 5600 rpm. Cells were washed with 100 µl Phosphate saline buffer (0.2mol/ml Na2HPO4, 0.2 mol/ml NaH2PO4, 0.8% (w/v) NaCl) and again centrifuged for 7 minutes at 5600 rpm. The pellet was resuspended in 150 µl TEGL buffer (25mmol/ml Tris pH 7.5, 10mmol/EDTA pH 8.0, 0.9% (w/v) Glucose, 10 mg/ml Lysozyme) and incubated for 3 hrs at 37℃ (Du Toit et al, 2001). Then the pellet was dissolved in 100µl of reducing buffer and denatured by heating in a boiling water bath for 10 min and then immediately transfer into ice and stored at -20℃.

**SDS-PAGE** The protein samples of S.aureus treated with LAB isolates were carried out in discontinuous buffer system (Berber et al, 2002) having 12% of resolving gel and 5% of stacking gel.

The gel was run at 45 V initially for 15 minute and then 100 V until the bromophenol blue marked had reached the bottom of the gel. Gels were stained with commassie brilliant blue. (Figure 2 & Figure 3).

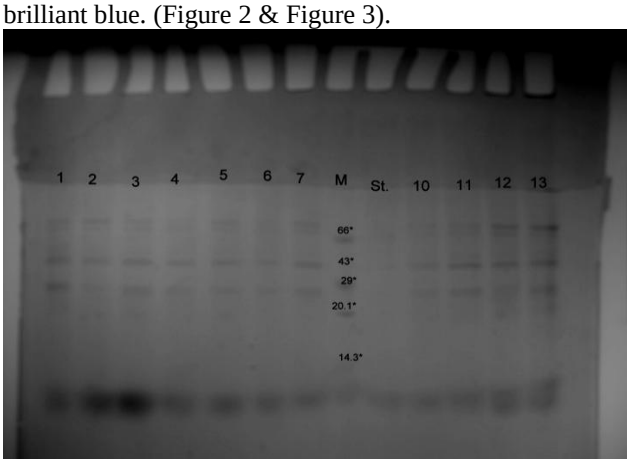



**Figure 2. SDS – PAGE of proteins of S. aureus treated with LAB isolates at different cell conc. Lanes: (1) S8 Pediococcus at 4.8×107 CFU/ml (2) S11 (Pediococcus) at 4.8×107 CFU/ml (3) S4 (Lactococcus) at 4.8×107 CFU/ml (4) S10 (Pediococcus) at 4.8×107 CFU/ml (5) S3 (Lactococcus) at 6×107 CFU/ml (6) S2 (Pediococcus at 6×107 CFU/ml (7) S5 (Leuconostoc) at 4.8×107 CFU/ml (8) Protein Molecular Marker ranging from 14.3 - 97.4 kD (9) Standard S. aureus (MTCC 3381) (10) S9 (Pediococcus) at 4.8×107 CFU/ml (11) S13 (Pediococcus) at 6×107 CFU/ml (12) S12 (Homofermentative Lactobacillus) at 4.8×107 CFU/ml .**

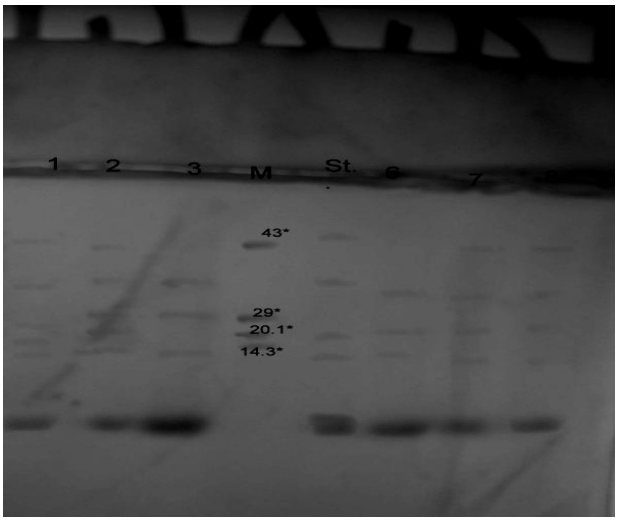



**Figure 3. SDS – PAGE of proteins of S. aureus treated with LAB isolates at different cell conc. Lanes: (1) S6 (Heterofermentative Lactobacillus) at 6 ×107 CFU/ml (2) S7 (Pediococcus) at 6×107 CFU/ml (3) S 1 (Lactococcus) at 0.66×107 CFU/ml (4) Protein Molecular weight marker rangingfrom 14.3 - 97.4 kD (5) Standard S.aureus (MTCC 3381) (6) S12 (Homofermentative Lactobacillus) at 0.53×107 CFU/ml (7) S13 (Pediococcus) at 0.66×107 CFU/ml (8) S2 (Pediococcus) at 0.66×107 CFU/ml.**

**Data Analysis** Bands were visualized and their molecular weights were determined by UN-SCAN-IT version 6.1. Protein marker weight of 14.3 – 97.3 kD was used as reference.

## Results and Discussion

A total of 13 LAB isolates, 3 (23%) belonged to Lactococcus, 7 (54%) to Pediococcus, 2 (15%) to Lactobacillus, 1 (8%) to Leuconostoc, which has shown in figure 4. The antimicrobial activity of LAB isolates at different dilutions was tested against S.aureus which has shown in figure 5 and figure 6. Out of 6 isolates of LAB having 15 × 108 CFU/ml, S1 gave largest zone of inhibition followed by S13 (Pediococcus), S2 (Pediococcus) and S7 (Pediococcus) at 6 × 107 CFU/ml, while only 3 isolates S1(Lactococcus), S2 (Pediococcus) and S13 (Pediococcus) also gave inhibitory zones at 2 × 107CFU/ml and 0.66 × 107 CFU/ml. On the other hand, out of 7 LAB isolates having 12 × 108 CFU/ml, S5 (Leuconostoc) and S8 (Pediococcus) gave largest zone of inhibition at 4.8 × 107 CFU/ml. These zones are followed by S12 (Homofermentative Lactobacillus) only gave zone of inhibition at 0.53 × 107 CFU/ml.These zones are followed by S12 (Homofermentative Lactobacillus), S10 (Pediocoocus) S11 (Pediococcus) and S9 (Pediocoocus).While S12 (Homofermentative Lactobacillus) only gave zone of inhibition at 0.53 × 107 CFU/ml. These results may be because of the wide spectrum of antimicrobial activities of LAB isolates which varies according to the serotype (Savodogo et al., 2004). The results depicted that inhibition zones decreases as the concentration of LAB isolates. Out of the 13 isolates, S1 (Lactococcus), S2 (Pediococcus), S13 (Pediococcus) and S12 (Lactobacillus) gave lowest minimum inhibitory concentration of 0.66 × 107 CFU/ml and 0.53 × 107 CFU/ml which has shown in figure 7. These 4 LAB isolates indicate that they are highly sensitive in inhibiting the growth of S.aureus even at a low concentration.

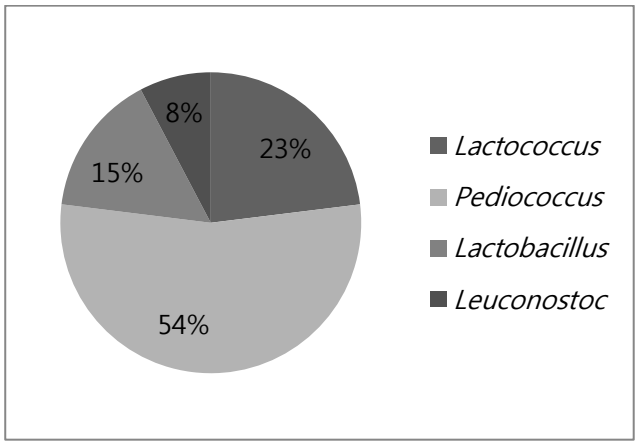



**Figure 4. Percentage of Confirmed isolates of LAB at genera level**

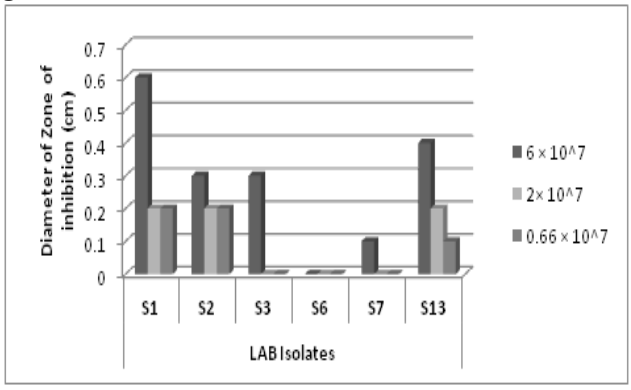



**Figure 5. Zone of inhibition produced by LAB isolates (15×10$^8$CFU/ml) against the standard strain of *S.aureus* (MTCC 3381)**

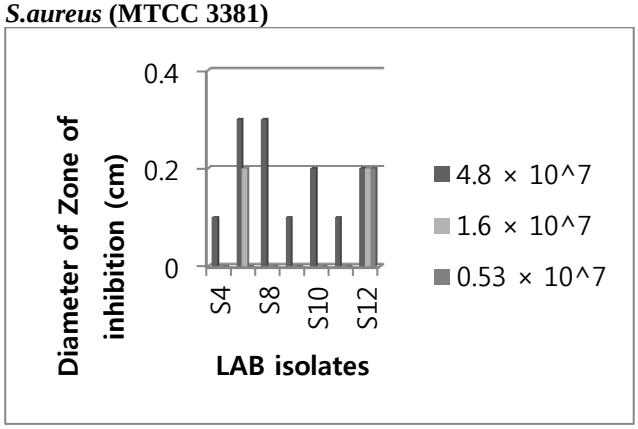
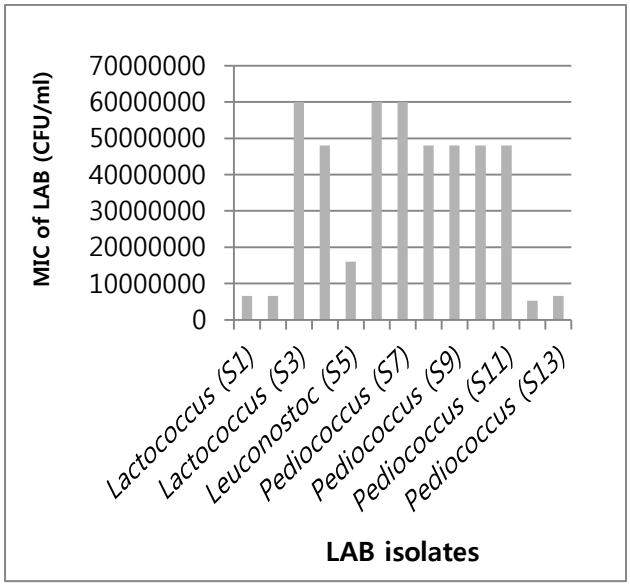



**Figure 6. Zone of inhibition produced by LAB isolates (12 ×108CFU/ml) against the standard strain of S. aureus (MTCC 3381)**




**References**


Ammor S, Tauveron G, Dufour E, Chevallier I. 2006. Antibacterial activity of Lactic acid bacteria against spoilage and pathogenic bacteria isolated from the same meat small-scale facility. 1. Screening and characterization of the antibacterial compounds. Food control. 17: 454-461.

Al-Allaf MAH, Al- Rawi AMM, Al- Mola AT. 2009. Antimicrobial Activity of Lactic Acid bacteria isolated from minced-beef meat against some pathogenic Bacteria. Iraqi J. of Bact. Sci. 23(1): 115-117.

Berber I, Cokmus C, Atalan E. 2002. Characterization of *Staphylococcus spp*. By SDS-PAGE of whole – cell and extracellular proteins. Microbiol. 72(1): 42-47.

Brinkten B, Minekns M, Vandervossen, JMBM, Leer RJ, Huis in't Veld, JHJ. 1994. Antimicrobial activity of *Lactobacilli:* preliminary characterization and optimization of production of acidocin B, a novel bacteriocin produced by *Lactobacillus acidophilus* M46. J. Appl. Microbiol. 77(2): 140-148

DuToit M, Dicks LMT, Holzapfel, WH. 2001. Taxonomy of obligately homofermentative and facultatively heterofermentative *Lactobacilli* in pig faeces. Lett. Appl. Microbiol. 32: 199-204.

Millette M, Luquet FM, Lacroix M. 2006. *In vitro* growth control of selected pathogens by *Lactobacillus acidophilus* and *Lactobacillus casei* fermented milk. Lett. Appl. Microbiol. 44: 314-319.

Nostro A, Cannatelli MA, Musolino AD, Procopio F, Alonzo V. 2002. *Helichrysum italicum* extract interfaces with the production of enterotoxins by *Staphylococcus aureus*. Lett. Appl. Microbiol. 35: 181-184.

Savodogo A, Ouattara CAT, Bassole IHN, Traore AS. 2004. Antimicrobial activities of Lactic acid Bacteria strains isolated from Burkina Faso Fermented Milk. Pak. J. Nut. 3(3): 174-179.