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Isolation and Characterization of Probiotic *Lactobacillus* spp. from Fermented Food Sources

Shilpa Sabnis and Neha Pawar*

Department of Microbiology, the Institute of Science, Mumbai-400 032, India
**Corresponding author*

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A B S T R A C T

Probiotic bacteria can be used as a therapeutic strategy to ameliorate human diseases. This has led to the increase in the use of probiotics as functional foods. Lactic acid bacteria are one of the major strains to be used as probiotics. Therefore, the present study was aimed at isolating, identifying and characterizing lactobacilli from fermented food sources viz. curd and idli batter in terms of their suitability as probiotics as well as antibiotic sensitivity. Both the isolated lactobacilli viz. LAB C (from curd) and LAB IB (from idli batter) were found to show tolerance to bile salts (upto 1.5 %) and could tolerate acidic conditions (low pH values of pH 3) for upto 3h. On performing antibiotic susceptibility test using Disc Diffusion Method, both LAB C and LAB IB showed low level resistance resistance to all antibiotics used in the study viz. Nalidixic acid, Nitrofurantoin, Cephalothin, Ampicilin, Co-trimaxazole except Norfloxacin. On the other hand they themselves were found to demonstrate a broad spectrum activity towards the pathogens viz. *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Proteus*, *Candida albicans*, *Salmonella paratyphi B* used in the study thus suggesting the possibility of their use as live biotherapeutic probiotic agents.

Introduction

Over recent decades, the development and consumption of functional probiotic foods has been increasing alongside awareness of their beneficial effects in promoting gut health as well as in disease prevention and therapy, and this has raised interest in health-promoting foods. Major probiotic mechanisms of action include enhancement of the epithelial barrier, increased adhesion to intestinal mucosa, and concomitant inhibition of pathogen adhesion, competitive exclusion of pathogenic microorganisms, production of anti-bacterial substances and modulation of the immune system (Bermudez-Brito *et al.*, 2012). Probiotic microorganisms can shape the immune system both at the local and systemic level, which will allow future probiotics as treatments for many diseases.

Recent scientific work on the properties and functionality of living micro-organisms in food have suggested that probiotics play an important role in immunological, digestive and respiratory functions, and that they could have a significant effect on the alleviation of infectious diseases in children and other high-risk groups (Gogineni *et al.*, 2013).

To colonize the gastrointestinal tract, probiotic strains need to be ingested as large

populations and on a daily basis. Therefore, food manufacturers are trying to include probiotic strains in foods and beverages which are part of a normal diet to provide health defense while enjoying meals and to differentiate such functional products from concentrated probiotic preparations available as capsules, powders, or liquids (Bermudez-Brito *et al.,* 2012).

Most probiotic-containing food products use lactic acid bacteria that have a long history of safe use in foods. As long as the strain is devoid of any transferable antibiotic resistance genes, members of the genera *Lactococcus*, *Lactobacillus*, and *Bifidobacterium* are considered safe; infections in humans by these genera are extremely rare (Sanders *et al.,* 2007). Probiotic lactic acid bacteria are often found naturally in foodstuffs such as milk, meat and vegetables (Setyawardani *et al.,* 2011).

Therefore, in the present study, an attempt was made to isolate and identify probiotic lactobacilli from milk derived products as well as fermented foodstuffs like idli batter and curd.

Materials and Methods

Isolation and identification of lactic acid bacteria

The lactobacilli from curd and idli batter were isolated sterile de Man Rogossa and Sharpe (MRS medium) anaerobically at 37°C/ 48 h.

The isolates were identified on the basis of their colonial, morphological and biochemical characteristics using standard microbiological techniques on comparison with Bergey's Manual of Determinative Microbiology. Their identity was confirmed using Biomérieux Vitek 2 Compact SYSTEM. The isolates were maintained at 4^0C.

Characterization of probiotic properties of *Lactobacillus* isolates

Determination of their resistance to low pH

Isolated cells were harvested from MRS broth by centrifugation (2000 rpm/ 10 min at 4°C). The pellet was washed with sterile Phosphate Buffered Saline (pH 7.2) and the cells were re-suspended in sterile PBS (pH 3).

Tubes were incubated at 37°C. Aliquots were taken after 0, 1, 2, and 3 h respectively and inoculated in sterile MRS broth followed by incubation at 37ºC anaerobically for 48h. Results were estimated in terms of its turbidity ensuring its growth which was further confirmed spectrophotometrically.

Determination of their resistance to bile salt

Overnight cultures of the isolates were independently inoculated into sterile MRS broth containing 0.3% and 1.5% of bile salt respectively. After 2h, a viable count was carried out and plates were incubated at 37°C for 48h anaerobically (Gotcheva *et al.,* 2002).

Determination of their resistance to antibiotics using disc diffusion method (Bauer *et al.,* 1966)

Overnight culture of the isolates was seeded into sterile MRS Agar. Hexadiscs containing various antibiotics viz. Nalidixic acid (NA), Nitrofurantoin (NIT), Cephalothin (CEP), Ampicilin (AMP), Co-trimoxazole (COT), Norfloxacin (NX) were placed on this seeded MRS agar and the plates were incubated at 37ºC anaerobically for 24h.

The sensitivity of the isolates to various antibiotics was recorded in terms of the zones of inhibition obtained around the antibiotic discs (Hummel *et al.,* 2007).

Determination of their antimicrobial activity on bacterial pathogens using agar cup method

The antimicrobial activity of the cell free supernatant (CFS) obtained from MRS broth containing LAB incubated at 37°C/ 48h followed by centrifugation at 5000 rpm /15min was checked using Agar Cup Method. Eighteen hour old cultures of bacterial pathogens (viz. *Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae,, Proteus, Candida albicans, Salmonella paratyphi B* (Culture density 10^8 cells/ ml) were individually seeded into sterile Nutrient Agar. Wells (0.6mm) were bored with help of cork borer to which the CFS was added. Plates were incubated at 37°C for 24h. The sensitivity of the isolates was recorded in terms of the zones of inhibition obtained around the wells.

Results and Discussion

Identification of isolates

Presumptive identification of the isolates was carried out on the basis of biochemical tests after comparing with Bergey's manual and with the help of Biomérieux Vitek 2 Compact SYSTEM, with anaerobic cards which is basically an automated microbiology system utilizing growth-based technology. All the isolates from idli batter and curd were found to be catalase negative, non-spore forming, non-motile, Gram positive and oxidase negative bacilli fermenting sugars without gas formation. The isolates were identified to belong to *Lactobacillus spp.*

Characterization of probiotic properties of *Lactobacillus* isolates

Determination of their resistance to low pH

Being able to tolerate acidic stress is one of the major selection criteria for probiotic strains. The probiotic bacteria are normally subjected to unfavorable physiological conditions viz. acidic environment and bile secretions of the Gastrointestinal (GI) tract. Viability of these bacteria upon ingestion and sufficient survival through the transit to GI tract is crucial for conferring any health benefits to the host. Survival at pH 3 is a significant feature, as ingestion with food lowers the pH in the stomach to 3 or even lesser (Kumar *et al.,* 2015). In the present study, the isolates were tested for their survival at pH 3.0 for 1, 2, 3 and 4 h. respectively. Both the *Lactobacillus* isolates were found to survive in the acidic environment for a period upto 4h (Figure 1). The general time taken for the process of digestion in the stomach is 3h (Hawaz, 2014). Both the isolates satisfy the basic requirement of tolerance of acidic stress. The results of our study are thus at par with earlier reports (Mourad and Eddine, 2006; Hawaz, 2014) and can be suitably tested further for their probiotic characteristics.

Determination of their resistance to bile salt

Tolerance to bile salts is considered to be another prerequisite for colonization and metabolic activity of bacteria in the small intestine of the host. Therefore, when evaluating the potential of using lactic acid bacteria as effective probiotics it is generally considered necessary to evaluate their ability to resist the effects of bile acids (Mourad and Nour-Eddin, 2006). The concentrations of bile salt used for the testing of the stains in the present study (viz. 0.3% and 1.5%) represents the extreme concentration obtained in human intestine during the first hour of digestion (Gotcheva *et al.,* 2002) after which, the normal level of bile salt in the intestine is around 0.3%. The resistance to bile salts varies from one lactic acid bacterial spp. to another and even between strains themselves. Bile resistance of some strains is related to

specific enzyme activity-bile salt hydrolase (BSH) which helps hydrolyse conjugated bile, thus reducing its toxic effect. In the present study, though both the isolates tolerated 0.3% as well as 1.5 % bile salts, the lactobacilli isolated from Idli batter (LAB IB) showed better tolerance to 0.3% and 1.5% concentration of bile as compared to lactobacilli isolated from Curd (LAB C) (Figure 2).

Determination of their resistance to antibiotics

The presence of transmissible antibiotic resistance markers is thus an important safety criterion for the evaluation of strains to be used as probiotics. Bacteria used as starter cultures for the production of foods could possibly contain antibiotic resistance genes. But intrinsic resistance to some antibiotics is inherent to the physiology of certain probiotic strains because of their cell wall structure or other inherent physiological characteristics.

The expression of resistance to an antibiotic is considered a risk factor if strains are suspected of harboring acquired, transferable antibiotic resistance genes, as suggested by expression of resistance to an antibiotic that exceeds the range determined to be normal for the species. In many instances, resistance to antibiotics is not transmissible, and the species also are sensitive to many other clinically used antibiotics. No particular safety concern, therefore, is associated with an intrinsic type of resistance. Therefore, in the current study, on performing antibiotic susceptibility test using Disc Diffusion Method, both LAB C and LAB IB though sensitive to Norfloxacin, showed resistance to all other antibiotics used in the study viz. Nalidixic acid, Nitrofurantoin, Cephalothin, Ampicilin, Co-trimaxazole (Table 1). These results concur with WHO reports which state the resistance of *Lactobacillus* to various classes of antibiotics such as β-lactam ring and aminoglycoside group. The lactobacilli strains having resistance to antibiotics might be useful in co-administration with antibiotics in the treatment of intestinal disorders and/or in preventing antibiotic-induced diarrhea, in situation when resistance genes present in the probiotic strains are silent (Hummel *et al.*, 2014). This property of Lactobacillus against some antibiotics would thus, in fact be useful for preventive and therapeutic purpose in controlling intestinal infection.

Fig.1 Graph depicting survival of isolates at pH 3

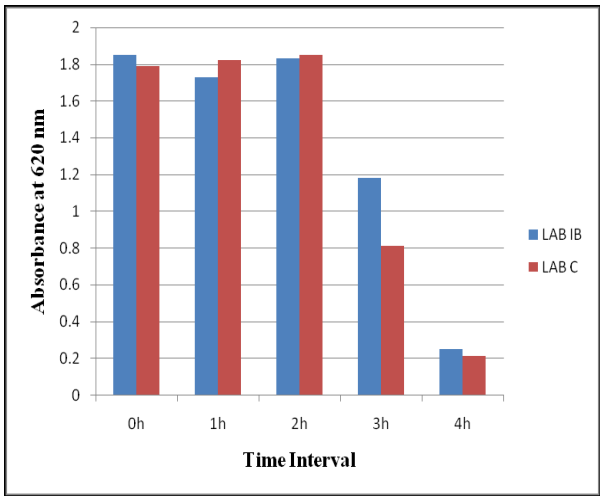

Fig.2 Graph depicting survival of isolates in presence of 0.3% and 1.5% bile salts

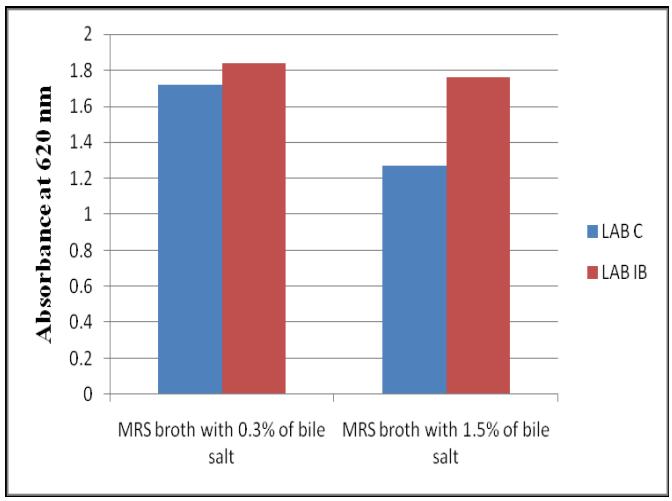

Fig.3 Antimicrobial activity of *Lactobacillus* isolates

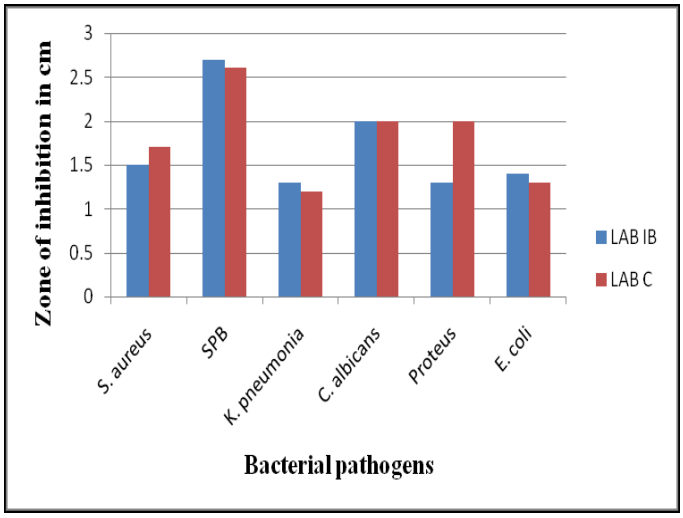

Table.1 Antibiotic sensitivity of the *Lactobacillus* isolates

Isolates	Nalidixic acid	Norfloxacin	Nitrofurantoin	Cephalothin	Ampicilin	Co-trimaxozole
LAB C	R	S	R	R	R	R
LAB IB	R	S	S	R	R	R

KEY: R: RESISTANT; S: SENSITIVE

Furthermore, the chances of transferring low level of resistance (intermediate susceptibility) are limited because such resistance is intrinsic and not plasmid mediated, and thus, the strains are safe to be used as probiotics (Halder and Mandal, 2015).

Determination of their antimicrobial activity on bacterial pathogens

The antimicrobial effect of lactic acid bacteria has been appreciated by man since ancient times and has enabled him to extend the shelf

life of many foods through fermentation processes. Innovative approaches have been tried as alternative to antibiotics in treating gastrointestinal diseases and these include using live biotherapeutic agents such as probiotic bacterial isolates. Lactic acid bacteria exert strong antagonistic activity against many microorganisms, including food spoilage organisms and pathogens. The benefits derived from a regular intake of probiotic foods are also correlated to their ability to inhibit pathogens and protect humans from gastrointestinal diseases (Lavermicocca *et al.,* 2005).

Lactic acid bacteria produce various compounds such as organic acids, diacetyl, hydrogen peroxide, and bacteriocin or bactericidal proteins during lactic fermentations The bacteriocins from the generally recognized as safe (GRAS) lactic acid bacteria (LAB) are now being used as a novel approach to control pathogens in food-stuffs.

In the present study as well, both the *Lactobacillus* isolates (LAB C and LAB IB) were found to show antibacterial activity against various pathogens used in the study viz. *Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae, Proteus, Candida albicans, Salmonella paratyphi B.* Out of the two isolates of lactobacilli, LAB C was found to be more inhibitory towards *Staphylococcus aureus* and *Proteus* while LAB IB was more inhibitory towards *Salmonella paratyphi B, Klebsiella pneumoniae* and *Escherichia coli. Candida albicans* however was found to be inhibited to the same extent by both LAB C as well as LAB IB (Fig. 3). Thus, both the isolates showed a broad spectrum activity towards the pathogens used in the study. Based on all these characteristics, both the isolates of lactobacilli were found to be suitable candidates to be used as probiotic strains.

With newer strain-specific clinical trials and meta-analysis of the clinical trials, the beneficial role of probiotics in certain diseases has been evolving. Some uncertainity still exists with probiotics in other diseases with regard to the therapeutic role, strain-specificity, dosage and duration. Identification of clinical characteristics of effective probiotic strains, their mechanisms of action and testing of probiotic-based treatment may provide the true beneficial effect of probiotics in various disorders.

References

Anja S. Hummel, Christian Hertel, Wilhelm H. Holzapfel, and Charles M. A. P. Franz1 2007. Antibiotic Resistances of Starter and Probiotic Strains of Lactic Acid Bacteria. *Applied and Environmental Microbiology,* 73, pp: 730–739.

Bathal Vijaya Kumar, Mannepula Sreedharamurthy, Obulam Vijaya Sarathi Reddy, 2015. Probiotication of Mango and Sapota Juices using *Lactobacillus Plantarum* NCDC LP 20. *Nutrafoods*, 14 (2), pp: 97-106.

Bauer, A.W., W. M. M. Kirby, J. C. Sherris, and M. Turck 1966. Antibiotic Susceptibility Testing By a Standardized Single Disk Method. *American Journal of Clinical Pathology*, 45:493–496.

Debashis Halder, Shyamapada Mandal, 2015, Curd Lactobacilli with probiotic potentiality, *Translational Biomedicine*, 6 (2), pp: 1-6.

Hawaz, E., 2014. Isolation and identification of probiotic lactic acid bacteria from curd and in vitro evaluation of its growth inhibition activities against pathogenic bacteria. *African J. Microbiol. Res*., 8(13), pp: 1419-1425.

Miriam Bermudez-Brito Julio Plaza-Díaz Sergio Muñoz-Quezada Carolina

Gómez-Llorente Angel Gil, 2012. Probiotic mechanism of action. *Ann Nutr Metab.*, 61, pp: 160–174.

Mourad, K., and Nour-Eddine, K. 2006. In vitro pre-selection criteria for probiotic lactobacillus plantarum strains of fermented olives origin. *Int. J. Probiotics Prebiotics*. 1, pp: 27-32.

Paola Lavermicocca, Francesca Valerio, Stella Lisa Lonigro, Maria De Angelis, Lorenzo Morelli, Maria Luisa Callegari, Carlo G. Rizzello,and Angelo Visconti 2005. Study of Adhesion and Survival of *Lactobacilli* and *Bifidobacteria* on Table Olives with the Aim of Formulating a New Probiotic Food. *Appl. Environ. Microbiol*, 71(8), pp: 4233–4240.

Sanders, M.E., Gibson, G.R., Gill, H.S. and Guarner, F. 2007. Probiotics: Their Potential to Impact Human Health. Council for Agricultural Science and Technology Issue paper (36), pp: 1-20.

Setyawardani, T., Rahayu, W.P., Maheswari, R., Palupi, N.H.S. 2011. Identification and Characterization of Probiotic Lactic Acid Bacteria Isolated from Indigenous Goat Milk. *Ani. Production*, 13(1), pp: 57–63.

Velitchka Gotcheva, Eli Hristozova, Tsonka Hristozova, Mingruo Guo, Zlatka Roshkova (2002), Assessment Of Potential Probiotic Properties Of Lactic Acid Bacteria And Yeast Strains, Food Biotechnology, 16 (3), pp: 211-225.

Vijaya K. Gogineni, Lee E. Morrow and Mark A Malesker 2013. Probiotics: Mechanisms of Action and Clinical Applications, J Prob Health, 1(1), pp: 1-11.

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