



E-ISSN: 2278-4136

P-ISSN: 2349-8234

JPP 2019; 8(5): 2154-2157

Received: 07-07-2019

Accepted: 09-08-2019

AS Moses

Associate Professor,
Department of Botany, Ewing
Christian College, Prayagraj,
Uttar Pradesh, India

ADM David

Associate Professor,
Department of Chemistry, Ewing
Christian College, Prayagraj,
Uttar Pradesh, India

Jane C Benjamin

Assistant Professor, Department
of Industrial Microbiology, Sam
Higginbottom University of
Agriculture, Technology and
Sciences, Prayagraj,
Uttar Pradesh, India

Sakshi Pandey

Department of Botany,
Ewing Christian College,
Prayagraj, Uttar Pradesh, India

Corresponding Author:**Jane C Benjamin**

Assistant Professor, Department
of Industrial Microbiology, Sam
Higginbottom University of
Agriculture, Technology and
Sciences, Prayagraj,
Uttar Pradesh, India

Isolation and identification of antagonistic *Lactobacillus acidophilus* from curd against MDR enteric pathogens

AS Moses, ADM David, Jane C Benjamin and Sakshi Pandey

Abstract

The present study is directed towards isolation and identification of antagonistic *Lactobacillus acidophilus* obtained by curd collected from local market of Prayagraj. After careful examination of cultural, morphological and biochemical characteristics, six strains of *Lactobacillus acidophilus* namely LA1, LA2, LA3, LA4, LA5 and LA6 were isolated. Antimicrobial activity of these six strains were evaluated by agar well diffusion method against *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli* and *Salmonella typhi*. Cell free supernatant obtained from the isolated strains exhibited maximum antimicrobial activity against selected pathogens and the result reveals that strains of *Lactobacillus acidophilus* have a good antagonistic property and thus revealing that curd is safe and beneficial for consumption.

Keywords: Antimicrobial activity, curd, enteric pathogens, *Lactobacillus acidophilus*

Introduction

Lactic acid bacteria are a group of gram positive bacteria, non- pathogenic, non-spore forming, catalase negative, cocci or rod shaped frequently isolated from fermented dairy products and milkable to produce lactic acid as the end product of carbohydrate fermentation. Lactic acid bacteria attained major attention for their widespread use in the production of fermented foods due to having probiotic qualities (Savadojo *et al.*, 2006) [5]. They are used as starter culture in fermentation and some of them are natural components of intestinal microflora. Lactic acid bacteria especially *Lactobacilli* considered as potential probiotic, various strains of *Lactobacilli* are used as health promoting factors. *Lactobacilli* maintains the natural microflora of the body and also protects the human bodies from various disease causing bacteria. *Lactobacilli* represents a significant part of our intestinal microflora and their friendship with general state of human health is under investigation. The genus *Lactobacilli* is one of the major group of Lactic acid bacteria and is of great economic importance. Lactic acid bacteria have been receiving considerable attention as probiotics because of their innate ability to exert antagonistic activity. Appreciable number of research have been done on *Lactobacilli* with emphasis on their health promoting properties and mode of antimicrobial action. Strains of *Lactobacillus acidophilus* were introduced in the dairy products because of the potential advantages through various products delivering of probiotics to human are being practiced dietary supplements, dairy products such as curd, ice cream, cheese and other fermented products. *Lactobacillus acidophilus* gets its name from lacto= milk bacillus = rod- like in shape and acidophilus stands for acid loving. It occurs naturally in human and animals and produces only lactic acid. Curd is traditional fermented milk product and is a very popular menu at the end of the meal in India. Curd is a rich source of Lactic acid bacteria, due to availability of raw milk, easy manufacturing process, low cost and high nutritive value make curd as one of the popular food items in Indian subcontinents. By acknowledging curds suitability as dietary agent of providing probiotics to intestine due this property of curd in the recent year much attention is given to isolation of *Lactobacilli* from different sources. In order to provide health benefits by *Lactobacilli* present in curd they required relevant characterization and identification. The present study has been carried out with objective to screen identify and characterize *Lactobacilli* from locally available curd to study their antimicrobial activities due to presence of probiotic properties. Therefore, the present study was carried out to evaluate antimicrobial values of curd with respect to the presence of antagonistic *Lactobacillus acidophilus*.

Materials and Methods

Place of work: The present study entitled “Isolation and Identification of Antagonistic *Lactobacillus acidophilus* from curd against MDR enteric pathogens” was carried in the Centre for Microbiology, Department of Botany, Ewing Christian College, Prayagraj.

Study Sample: Fresh curd from local market was used for the isolation of *Lactobacillus* spp. The antimicrobial activity was studied against four preisolated MDR enteric bacterial pathogens viz. *S. aureus*, *B. cereus*, *S. typhi* and *E. coli*. These pathogenic bacteria were procured from Microbial Culture Collection Bank, Department of Industrial Microbiology, Sam Higginbottom University of Agriculture, Technology and Sciences, Prayagraj.

Collection of curd sample

Curd sample was collected from a local shop of Allahabad city. For this fresh good quality curd (50gm) was purchased from a local sweet shop and was kept in sterilized glass bottles. After collection of the curd sample it was immediately transported to the laboratory and analyzed.

Isolation and maintenance of *Lactobacillus acidophilus*

The initial isolation of *Lactobacillus acidophilus* strains from curd was done on de Mann Rogosa Sharpe (MRS) medium. For this 1gm curd was taken and through serial dilution pour plate technique 1ml curd was transferred on the MRS medium and then these plates were kept in desiccators to provide anaerobic conditions through candle jar extension method and incubated at 37°C for 24hrs. After complete incubation obtained colonies were subcultured and were subjected to various identification parameters.

Identification of isolated *Lactobacillus acidophilus*

Lactobacillus strains isolated in the study was identified on the basis of cultural, morphological and biochemical characteristics.

Cultural characteristics

The colonies appeared on MRS plates were separate, creamish white, smooth, small round, flat, opaque with no pigmentation.

Morphological characteristics

It was done with the help of Gram staining (Holt *et al.*, 1994)^[9]. It was done for identification and classifying bacteria as gram positive or gram negative. The bacteria which retains primary crystal violet stain was named as gram positive, whereas those that lost primary stain and counter stain by safranin appears red were referred as gram negative.

Lactobacillus acidophilus is a gram positive bacteria hence it retains the primary crystal violet stain.

Biochemical characteristics

Catalase test: Due to presence of catalase enzyme, when colonies were treated with H₂O₂ they lack the production of H₂O and O₂.

The isolated and identified *Lactobacillus acidophilus* strains were inoculated in MRS broth and kept in desiccators for providing anaerobic conditions through candle jar extension method and kept in incubator for 24hrs at 37°C.

Antagonistic activity of *Lactobacillus acidophilus* against selected bacterial pathogens

It was done by agar well diffusion method on Nutrient Agar medium (Tagg and Mcgiuen, 1971)^[10]. Overnight (18hrs) broth culture of pathogen was swabbed uniformly on the surface of Nutrient Agar plates using sterilised cotton swabs. One plate of Nutrient Agar was kept as media control. For pathogen control 4 N.A plates were swabbed with 4 pathogens. For treatment control 8 N.A plates were swabbed by 4 pathogens, 4 wells were cut in 4 plates and 3 wells were cut in other 4 plates, the plates in which 4 wells were cut was named as LA1, LA2, LA3 and LA4 and the other plates in which 3 wells were cut was named as LA5, LA6 and TC (LA1, 2, 3, 4, 5, and 6 are the different strains of *Lactobacillus acidophilus* that was isolated and identified). In 6 wells 0.1ml of cell free extract of *Lactobacillus acidophilus* strains which was obtained by centrifugation (10min) was filled and in TC well only MRS broth of 0.1ml was filled. Plates were incubated at 37°C for 24-48hrs. Zone of inhibition formed around wells was observed and measured in millimetres and results were recorded (Bhowaland Chakraborty., 2015).

Results and Discussions

Isolation and identification of *Lactobacillus acidophilus* from the selected curd sample: Total six strains of *Lactobacillus acidophilus* were isolated from the curd sample which were Gram positive and catalase negative. They were identified on the basis of their colony characteristics appeared creamy white colored, circular with entire margin and morphological characteristics.

Antimicrobial activity of *Lactobacillus acidophilus*

The antagonistic activity of cell free supernatant of different *Lactobacillus acidophilus* strains were screened against or tested against four pathogenic bacteria that are *Bacillus cereus*, *Salmonella typhi*, *Staphylococcus aureus*, *Escherichia coli* and they showed different inhibitory actions by forming a inhibition zones (Table-1, Figure 1) by using well diffusion method.

Table 1: Antimicrobial activity of *Lactobacillus* isolates against tested bacterial pathogens

S. No.	<i>Lactobacillus acidophilus</i> strains	Zone of inhibition against pathogens (mm)			
		<i>Staphylococcus aureus</i>	<i>Bacillus cereus</i>	<i>Escherichia coli</i>	<i>Salmonella typhi</i>
1.	LA1	0.0	11.0	0.0	0.0
2.	LA2	0.0	0.0	0.0	0.0
3.	LA3	0.0	0.0	0.0	0.0
4.	LA4	0.0	13.0	0.0	0.0
5.	LA5	7.0	15.0	0.0	16.0
6.	LA6	0.0	0.0	0.0	5.0

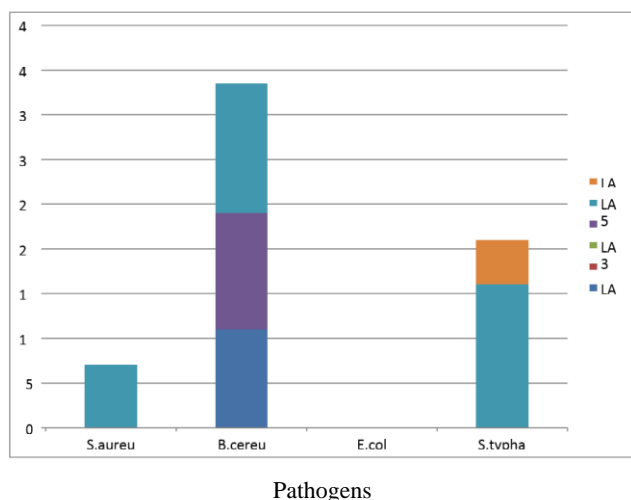


Fig 1: Antimicrobial activity of Lactobacillus isolates against tested bacterial pathogens

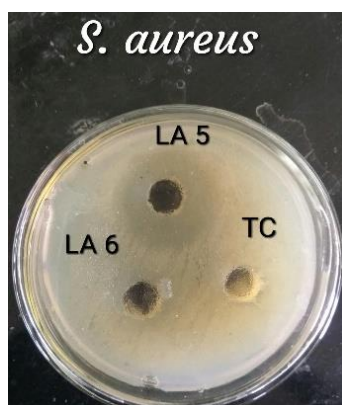


Plate 1: Antagonistic activity of Lactobacillus isolates against *Staphylococcus aureus*

Among these *Lactobacillus acidophilus* strains LA1 showed 11.0 mm inhibition zone against *Bacillus cereus*, and it does not showed any inhibition zone against other three pathogens whereas LA2 and LA3 showed zero inhibition zone against all the four pathogens. LA4 strain showed 13.0 mm inhibition zone against *Bacillus cereus*, 0.0 mm against *E. coli*, 0.0 mm against *S. aureus*, and 0.0 mm against *S. typhi*. LA5 showed 7.0 mm inhibition zone against *S. aureus*, 15.0 mm inhibition zone against *B. cereus*, 16 mm inhibition zone against *S. typhi* and showed 0.0 mm inhibition zone against *E. coli*. LA6 strain showed 5.0 mm inhibition zone against *S. typhi* but it does not showed any inhibition zone against the *S. aureus*, *B. cereus*, and *E. coli*. According to the data which was obtained by above experiment it is clear that among these different strains of *Lactobacillus acidophilus* isolates, LA5 was found to be the most antagonistic strain which showed inhibition activity against *B. cereus*, *S. aureus*, and *S. typhi*. while LA2 and LA3 strains has zero antagonistic activity against these pathogens. Among pathogens *Bacillus cereus* was most inhibited by *L. acidophilus* strains followed by *Salmonella typhi* and *Staphylococcus aureus*. But these strains failed to show inhibitory activities against *E. coli*. As *E. coli* is gram negative bacteria, it could be due to its complex cell wall structure than gram positive bacteria. In gram negative bacteria beside peptidoglycan, there are phospholipids, proteins and lipopolysaccharides in the cell wall. This could be a reason due to which these strains were failed to inhibit the growth of *E. coli*. According to Patra *et al.*, (2011)^[8] the inhibitory metabolites produced by isolated *Lactobacillus* species were extracellular and diffusible due to which they

were able to inhibit the growth of the microorganisms. Chowdhury and Islam (2016)^[2] documented several mechanisms that have been attributed to explain antagonistic activities of LABs. These mechanisms may be lowering pH of the harboring environment by producing lactic and acetic acid, Competition for nutrients and adhesion sites with other inhabiting bacteria in surroundings. Production of bacteriocins and antioxidants are some of the pronounced mechanisms of antimicrobial actions. Thakkar *et al.*, (2015)^[6] reported that lactic acid bacteria tolerate high salt concentrations as it allows the bacteria to begin metabolism, which produces acid that further inhibits the growth of undesirable micro organisms. According to Hawaz (2014)^[4] production of some antimicrobial compounds such as organic acids, short chain fatty acids and bacteriocins are responsible for the inhibition of the growth of pathogenic micro organisms. The antimicrobial effect of lactobacilli is linked to the production organic acid, hydrogen peroxide and antimicrobial peptides with a variable range of actions was observed by Cortes- Zavaleta *et al.* (2014)^[7]. According to Gupta *et al.*, (2017) the sensitivity of Gram-negative pathogens can be linked to their thin peptidoglycan cell walls and their susceptibility toward acidic metabolites.

Conclusion

The experimental result shows that the strains of *Lactobacillus acidophilus* isolated from the curd through serial dilution pour plate technique exhibits antimicrobial activity against four pathogens *Bacillus cereus*, *Salmonella typhi*, *Staphylococcus aureus* and *Escherichia coli*. The tested isolates showed inhibitory zone against these pathogenic bacteria. Among the strains of *Lactobacillus acidophilus* LA5 showed maximum inhibitory activity against these pathogenic bacteria. In conclusion, the present study showed that the curd contains *Lactobacillus acidophilus* with good antagonistic property, hence this study shows that the consumption of curd is safe and beneficial for the humans as it protects or provide resistance to the human body against these tested pathogens. But detailed study on their role in human body, bile tolerance, acid tolerance, resistance against proteolytic enzymes and gastric juice is needed to keep them in probiotic category. The above study shows that Indian curd are good for health and the consumption of curd is safe.

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