



## Isolation and Identification of Lactobacilli Bacteria from Raw Cow Milk in Local Region of Agra.

Kajal Alam<sup>1</sup> Ankur Goyal<sup>2</sup> and Jagriti Sharma<sup>3</sup>

<sup>1</sup>Department of Microbiology, School of Life Sciences, Dr .B.R. Ambedkar University, Khandari Campus, Agra, U.P. (India).

<sup>2</sup>Department of Microbiology, School of Life Sciences, Dr .B.R. Ambedkar University, Khandari Campus, Agra, U.P. (India).

<sup>3</sup>Department of Microbiology, S.N. Medical College, Agra- 282002, U.P (India)

\*Corresponding author: [microjagriti@gmail.com](mailto:microjagriti@gmail.com)

### Abstract

*Lactobacilli* play a major role in fermented dairy products and also contribute to the therapeutic aspects of human health. Raw milk sample was used in this study to isolate and identify the *Lactobacilli* and to find out the incidence of *Lactobacilli* Biochemical i.e. IMViC tests, Sugar fermentation test were used for identification of isolates of *Lactobacilli* from raw cow milk. *Lactobacilli* were used to evaluate their antimicrobial effect against common pathogens, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli* by using the disc diffusion method. The study was successful in isolation and identification from raw cow milk in local region of Agra. The result of the present study indicates that, raw cow milk is potential source of probiotic Lactobacilli.

**Keywords:** Cow milk, Identification, Isolation, Biochemical tests and Antimicrobial activity

### Introduction

Probiotics are microorganisms that are believed to provide health benefits when consumed. (Hill et al., 2014) The history of probiotics began with the history of man by consuming fermented foods that is well known Greek and Romans consume very much (Gismondo et al. 1999; Guarner et al. 2005). Probiotics are suggested as food to provide for the balance of intestinal flora (Holzapfel, et al. 1998). Probiotics are used for long times in food ingredients for human and also to feed the animals without any side effects. Also probiotics are acceptable because of being naturally in intestinal tract of healthy human and in foods. Probiotic can be found in dairy and non-dairy Products. The primary probiotics bacteria associated with dairy products have been *Lactobacilli*

*acidophilus*, *L. casei* and *Bifidobacteria*, raw cow milk may be a suitable source of potential probiotic microorganisms. Cow milk and its products are widely consumed in developing countries due to their therapeutic and nutritional values. Lactose maldigestion occur frequently in the general population (Marteau, 2001) Persons digestion and tolerance of lactose in yogurt then that contained in milk. Cow Milk is a pale liquid produced by the mammary glands of cow. It is the primary source of nutrition for infant mammals before they are able to digest other types of food. (Wouters et al., 2002). The present study deals with the isolation and identification of *Lactobacilli* from various milk samples obtained.

## Materials and Methods

### Collection of milk samples

The samples of cow's milk were collected from January to July 2017 to isolate and identify the naturally occurring lactobacilli bacteria from raw cow milk. A total of ten raw cow milk samples were collected from lactating cows in the surrounding area of the city Agra. Milk samples were collected in sterile bottle and brought to laboratory with icebox and then transported to be analyzed.

### Isolation and identification of *Lactobacillus*

Appropriate dilutions of the collected milk samples were made in normal saline and pour plated on TGYA (Tryptone glucose yeast extract ager) media and incubated at 37°C an aerobically for 24 to 48 hours. (Ramalingam ,C et al., 2011) At the end of 48 hours, when the colonies became predominant, morphologically distinct and well isolated colonies were picked and transferred to new TGYA media plates by streaking. Colonies showing typical characteristics of *Lactobacilli* on medium surface were picked up randomly and transferred into TGYA broth for further enrichment. Further, their purity was checked on TGYA medium.

The pure isolates were subjected to identification as macroscopic appearance of all the colonies was examined for cultural and morphological characteristics. Size, shape, colour and texture of the colonies were recorded.

The isolates were stained by Gram's method and examined under microscope for purity and those isolates readily identified as Gram positive rods and catalase negative Oxidise negative were included for further characterization, production from carbohydrates (1 % w/v) - Lactose, in TGYA broth devoid of glucose and beef extract with phenol red as indicator; production of acid and gas from 1 % glucose (TGYA broth without beef extract); methyl red and Voges-Proskauer test in MRVP medium; production of ammonia from arginine; nitrate reduction in nitrate broth; indole production in tryptone broth and growth on acetate agar.(Aarti Bhardwaj et al., 2012), (Ghanbari M et al., 2009), (Anandharaj. M et al., 2013).

### Anibacterial activity of the isolates

Ager overlay method was used to determine the antibacterial activities of the isolated *Lactobacilli* Three different human pathogens belonging to both gram positive and gram negative groups such as *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC8739, *Pseudomonas aeruginosa* ATCC 27853 were used in this study as test pathogen. Antibacterial activity was further characterized by determining whether bacteriostatic or bactericidal. The test was performed by swabbing of the growth inhibition zone. The swab was streaked onto Muller Hinton ager plate and incubated aerobically at 37°C for 24 hours. The presence of growth in Muller Hinton ager plate was interpreted as an inhibitory activity i.e. bacteriostatic, while no growth was interpreted as bacterical.

## Results and Discussion

The goal of this work was to isolate and characterize potential probiotic bacteria from raw cow milk sample of Agra (Utter Pradesh) and to assess their anti-bacterial activity against some common pathogenic bacteria. Based on the morphology characteristics ten (10) isolate were identified as *Lactobacilli* from raw cow milk sample. After gram staining the isolated bacteria was rod shape, convex, rough, smooth, shiny, irregular, circular, gram positive, facultative anaerobic, non spore forming, which indicate them to be the member of *Lactobacilli*. A total of ten *Lactobacilli* isolates were characterized and identified from raw cow milk samples collected. Morphological, physiological and biochemical characteristics of isolated are shown in (Table 1, 2). All isolates were gram positive, non-motile, catalase negative, Oxidase negative, Indole test negative, MR-VP test positive, citrate test negative, glucose positive, lactose positive test.

Activity the selected strains were examined according to their antimicrobial activity. For this purpose, strains were detected against the indicator microorganisms *Escherichia coli* ATCC 8739, *Staphylococcus aureus* ATCC-25923 and *Pseudomonas aeruginosa* ATCC-27853. The diameter of inhibition zones showed that all of the isolates have antibacterial effect on the indicator microorganisms. The tests were applied five times and the averages of diameters of zones were given (Table 3).

**Table: 1** Morphology characterization of isolated *Lactobacilli* bacteria

S.No	Colony Morphology	Result
1.	Motility	Non motile
2.	Gram's reaction	Positive
3.	Cell shape	Rod
4.	Pigment	White-creamy
5.	Configuration	Round
6.	Spores	Negative
7.	Surface	Mucoid
8.	Elevation	Flat
9.	Texture	Dry
10.	Colony size	0.1-0.5mm

**Table: 2** Biochemical tests of isolated *Lactobacilli* bacteria

S.No	Characteristic	Result
1.	Catalase	-Ve
2.	Oxidase	-Ve
3.	Indole test	-Ve
4.	Methyl Red test	+Ve
5.	Voges Proskauer	+Ve
6.	Citrate	-Ve
	<b>Suger Fermentation</b>	
7.	Glucose	+Ve
8.	Lactose	+Ve

+ = Positive, - = Negative

**Table: 3** Antagonistic activity of *Lactobacilli* against *Pesudomonas aeruginosa*, *E. coli*, and *Staphylococcus aureus*

S. No	Antibiotic/ Probiotic disc	Zone of diameter(in mm)														
		Sample 3			Sample 5			Sample 7			Sample 8			Sample 10		
		<i>P.aer.</i>	<i>E.coli</i>	<i>S.aer</i>	<i>P.aer</i>	<i>E.coli</i>	<i>S.aer</i>	<i>P.aer</i>	<i>E.coli</i>	<i>S.aer</i>	<i>P.ae</i>	<i>E.coli</i>	<i>S.ae</i>	<i>P.aer</i>	<i>E.coli</i>	<i>S.ae</i>
1	Antibiotic	18	24	19	18	21	24	20	24	18	20	23	18	16	21	18
2	1	9	12	8	9	9	11	10	12	8	10	19	8	9	8	8
3	1/10	7	8	5	8	7	7	6	8	5	9	17	5	5	5	4
4	1/100	3	5	2	3	0	4	2	4	0	0	9	0	3	2	0
5	Distilled water	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

**P.aer.** = *Staphylococcus aureus*, **E.coli**=*Escherichia coli*, **P.aer** = *Pesudomonas aeruginosa*

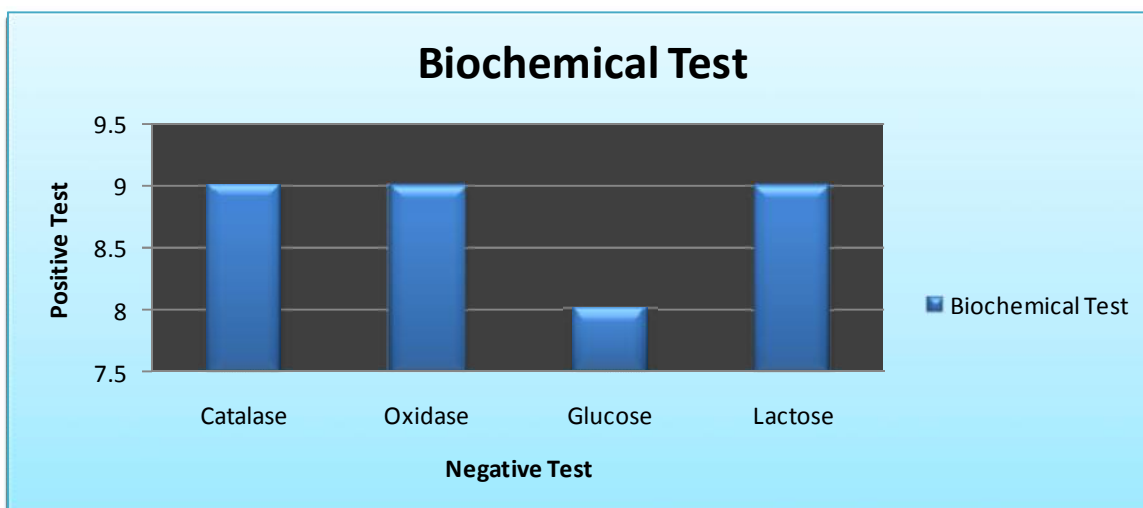


Figure 1. Biochemical test

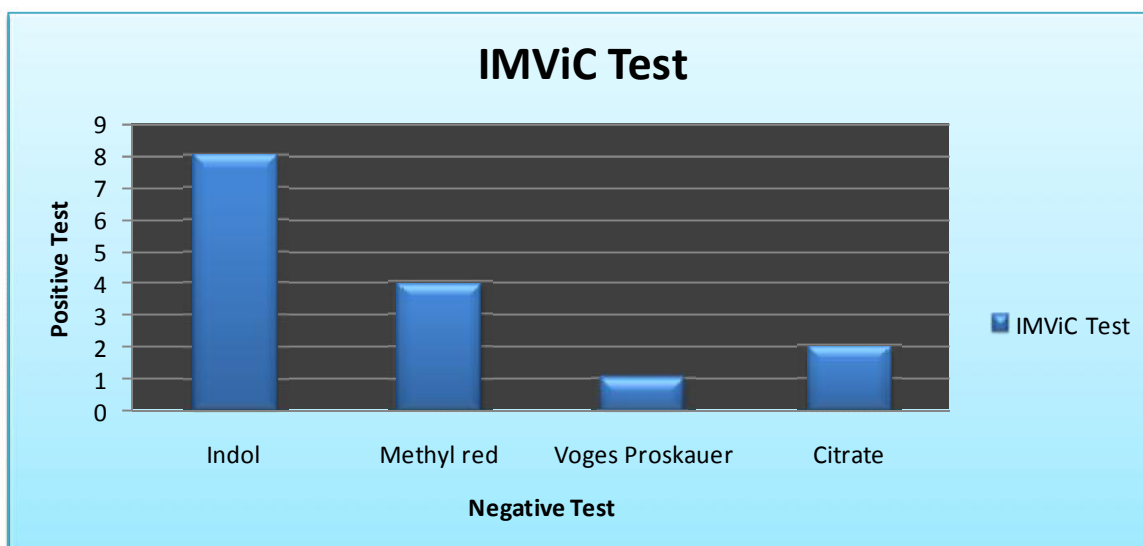


Figure 2. IMViC Test

Maximum inhibition of zone was produced by *Lactobacilli* against *E.coli* ATCC 8739(12mm) by serial suspension M.F.S 1.0, medium zone were observed in the serial suspension of M.F.S 1/10 followed by *Pseudomonas aeruginosa* ATCC-27853(10mm) and *Staphylococcus aureus* ATCC-25923 (8mm). By serial suspension M.F.S. 1.0, medium zone were observed in the serial suspension of M.F.S. 1/10 (8 mm to 5 mm) so for as 1/100 suspension is concerned a zone of 5 mm was seen against *E. coli* and *Staphylococcus aureus* 2 mm and *Pseudomonas aeruginosa* no zone.

## Conclusion

The results of this study indicate that cow milk exhibit a wide diversity of *Lactobacilli* occurring naturally in

the milk and can be used as a potential natural source to isolate a variety of strains of *Lactobacilli*. It is clear from the above that *Lactobacilli* produced better zone against *P.aeruginosa* and *S.aureus* but reverse was true against *E.coli*. It is clear from the above these strains may be used as the therapeutic agent for the various infections, specially against the resistant pathogen like *S. aureus* and *P.aeruginosa* etc Since some strains of *Lactobacilli* possess potential probiotic and therapeutic properties including anti-inflammatory and anti-cancer activities, as well as other features of interest, these isolates can be further screened for their probiotic and related properties and exploited for health and economic benefits.

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## References

- Aarti Bhardwaj, Monica Puniya, K. P. S. Sangu, Sanjay Kumar and Tejpal Dhewa.(2012). Isolation and Biochemical Characterization of Lactobacillus species Isolated from Dahi. *A Journal of Dairy Science and Technology Volume 1, Issue 2, pp18-31*
- Gismondo, M.R., Drago, L., Lombardi, A. (1999). Review of probiotics available to modify gastrointestinal flora. *International Journal of Antimicrobial Agents. 12:287-292.*
- Ghanbari M, Rezaei M, Jami M and Nazari, R. M. (2009). Isolation and characterization of Lactobacillus species from intestinal contents of beluga (*Huso huso*) and Persian sturgeon (*Acipenser persicus*). *Iranian Journal of Veterinary Research, Shiraz University, Vol. 10, No. 2.*
- Guarner, F., Perdigon, G., Corthier, G., Salminen, S., Koletzko, B. and Morelli, L. (2005). Should yoghurt cultures be considered probiotic. *British Journal of Nutrition. 93:783-786*
- Hill, C; Guarner, F; Reid, G; Gibson, GR; Merenstein, DJ; Pot, B; Morelli, L; Canani, RB; Flint, HJ; Salminen, S; Calder, PC; Sanders.(2014). “Expert consensus document. The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic.” *Nature Reviews. Gastroenterology & Hepatology. 11(8): 506- 14.*
- Holzappel, W.H., Haberer, P., Snell, J., Schillinger, U., Huis in’t Veld, J. (1998). Overview of gut flora and probiotics. *International Journal of Food Microbiology 41:85-101.*
- Marteau PR, DeVreseem, Cellier CJ. and Schrezenmeir J. (2001). Protection from gastrointestinal diseases with the use of Probiotics. *Am J. Clin. Nutr. 73: 4305-4365.*
- Ramalingam, C. and Anvita Karara. Isolation and characterization of bacteriocin producing Lactobacillus and bacteriocin from traditional fermented food. *International Journal of Microbiology and Bioinformatics - Vol.1, Issue, 1, November, 2011, pp.010-015.*
- Vamanu Emanuel, Vamanu Adrian, Popa Ovidiu and Câmpeanu Gheorghe(2005) .Isolation of a *Lactobacillus plantarum* strain used for obtaining a product for the preservation of fodders. *African Journal of Biotechnology Vol. 4 (5), pp. 403-408*
- Wouters J. T, Ayad E. H, Hugenholtz J, Smit G,(2002). “Microbes from Raw Milk for Fermented Dairy Products,” *International Dairy Journal, Vol. 12, No. 2-3, pp. 91-109.*

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