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Antagonistic Effect of *Lactobacillus* Isolates from cow Milk on selected Pathogenic Bacteria

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Abstract

The viable microbial food supplements which beneficially influence the health of humans are known as probiotics. Antibacterial activity is one of the important probiotic properties for a strain to be a functional probiotic. The three *Lactobacillus* strains namely *Lactobacillus plantarum* CM1, *Lactobacillus fermentum* CM4 and *Lactobacillus casei* subsp. *casei* CM6 were isolated from cow milk samples collected from Udaipur city. Their antagonistic activity was tested against five pathogenic bacteria such as *Serratia marcescens* NCDC 108, *Enterobactor aerogens* NCDC 106, *Proteus vulgaris* NCDC 73, *Pseudomonas aeruginosa* NCIM 5029, and *Micrococcus luteus* NCDC 131 using agar well assay method. The results showed that the *Lactobacillus* strains namely *Lactobacillus plantarum* CM1, *Lactobacillus fermentum* CM4 and *Lactobacillus casei* subsp. *casei* CM6 were able to inhibit the growth of all the pathogenic bacteria in varying degrees. All the three *Lactobacillus* isolates were found to be the most sensitive against *Micrococcus luteus* than the rest of the pathogenic bacteria used in the study. The inhibition observed in the case of all the isolates that have antagonistic effect may be due to the production of organic acids as well as other compounds such as bacteriocins, hydrogen peroxide etc.

Keywords: Probiotics, *Lactobacillus* isolates, antagonistic activity, pathogenic bacteria.

Introduction

Recent concerns on the rampant and indiscriminate use of antibiotics for disease treatments and growth promotion of livestock and the development of antibiotic resistant pathogens have led to increased interest in the application of probiotics and their antimicrobial metabolites as alternative antimicrobial strategies for treatment and prevention of infections. Hence, antimicrobial activity against pathogens is a desirable property of a potential probiotic strain[1]. Probiotics are defined as 'Live microorganisms, which when administered in adequate amounts confer a health benefits to the host[2]. Lactobacilli are known as probiotic bacteria due to beneficial health effects exerted by them. Lactobacilli have antimicrobial activity because of the production of organic acids (lactic and acetic acid), diacetyl, hydrogen peroxide, and bacteriocins e.g. acidocin, acidophilin, lacticin and nicin. Many researchers have been determined the antagonistic activity of lactobacilli against gram-positive and gram-negative pathogenic bacteria[3,4].

The present study was conducted to determine the antagonistic activity of *Lactobacillus* isolates against some pathogenic bacteria.

Material and Methods

The materials and methods used in the present study were as follows:

Maintenance and preservation of cultures: Lactobacillus strains: *Lactobacillus* isolates used in this study were isolated from cow milk samples, collected from various street venders and local dairies in Udaipur city. The isolates were stored in litmus milk at 10ºC. Cultures were activated in MRS broth with incubation at 37ºC for 24 h.

Pathogenic cultures: The pathogenic cultures used in this study were *Serratia marcescens* NCDC 108, *Enterobactor aerogens* NCDC 106, *Proteus vulgaris* NCDC 73, *Pseudomonas aeruginosa* NCIM 5029, and *Micrococcus luteus* NCDC 131. All the strains were obtained from National Dairy Research Institute, Karnal and NCIM, Chandigarh. The cultures were maintained on nutrient agar slant and activated in nutrient broth at 24h interval.

Detection of antagonistic activity: The isolates were screened for the antibacterial substance by inoculating active culture into MRS broth and incubating overnight at 37^0C. The stationary cells were centrifuged at 3000 rpm for 20 min. The culture supernatants neutralized with and without 1N NaOH were tested for antibacterial activities against different pathogenic bacteria. For determining the antibacterial activity of the isolates against the potential pathogenic bacteria agar well assay[5] was used. Finally the diameter of inhibition zone extending laterally around the well was measured with scale. The inhibition zone of more than 1 mm diameter was considered as positive inhibition.

Results and Discussion

Result: The antibacterial activity was determined in the two fractions of the cell free supernatants: normal cell supernatant

and cell supernatant neutralized with 1N NaOH. The normal cell supernatants of the three isolates showed higher antibacterial activities than the supernatants neutralized with NaOH.

The bacterial supernatants without NaOH of all the three *Lactobacillus* isolates *Lactobacillus plantarum* CM1, *Lactobacillus fermentum* CM4 and *Lactobacillus casei* subsp. *casei* CM6 showed highest inhibition zones against gram-positive indicator organisms (table 1 and figure 1). The sizes of inhibition zones obtained for *Lactobacillus plantarum* CM1, *Lactobacillus fermentum* CM4 and *Lactobacillus casei* subsp. *casei* CM6 against *Micrococcus luteus* were 19.5, 26.0 and 24.00 mm respectively. For *Lactobacillus plantarum* CM1 the sizes of inhibition zones against rest of the indicator organisms were ranged from 14.0 to 16.0 mm. For *Lactobacillus fermentum* CM4 and *Lactobacillus casei* subsp. *casei* CM6 the sizes of inhibition zones against rest of the indicator organisms were ranged from 9.5 to 18.0 mm and 11.0 to 20 mm respectively.

Table-1
Antibacterial activities of *Lactobacillus* isolates against different pathogenic bacteria (Without NaOH)

S. No.	Name of organism	Diameter of clear zone (mm) including well diameter					
		Serratia marcescens	***Enterobacter aerogenes***	***Proteus vulgaris***	***Pseudomonas aerouginosa***	***Micrococcus luteus***	**Mean**
1.	*Lactobacillus plantarum* CM1	16.0	14.0	15.0	16.0	19.5	16.1
2.	*Lactobacillus fermentum* CM4	10.5	14.0	09.5	18.0	26.0	15.6
3.	*Lactobacillus casei* subsp. *casei* CM6	13.0	15.0	11.0	20.0	24.0	16.6
4.	Mean	13.167	14.333	11.833	18.0	23.167	16.1

CRD ANOVA for Antibacterial activities of *Lactobacillus* isolates against different pathogenic bacteria (Without NaOH)

S. No.	Source	DF	SS	MS	F	SE(m)	CD(5)	CD(1)
1.	A	2	0.0499909	0.0249954	2.777	0.03	0.09043	0.1251
2.	B	4	5.00866	1.25217	139.130**	0.03873	0.1167	0.1615
3.	A x B	8	1.19334	0.149167	16.574**	0.06708	0.2022	0.2797
4.	Error	15	0.134999	0.00899994				

CV = 5.8924, A = *Lactobacillus* isolates, B = Indicator bacteria, *, ** Significant at 5 and 1 percent level of significance

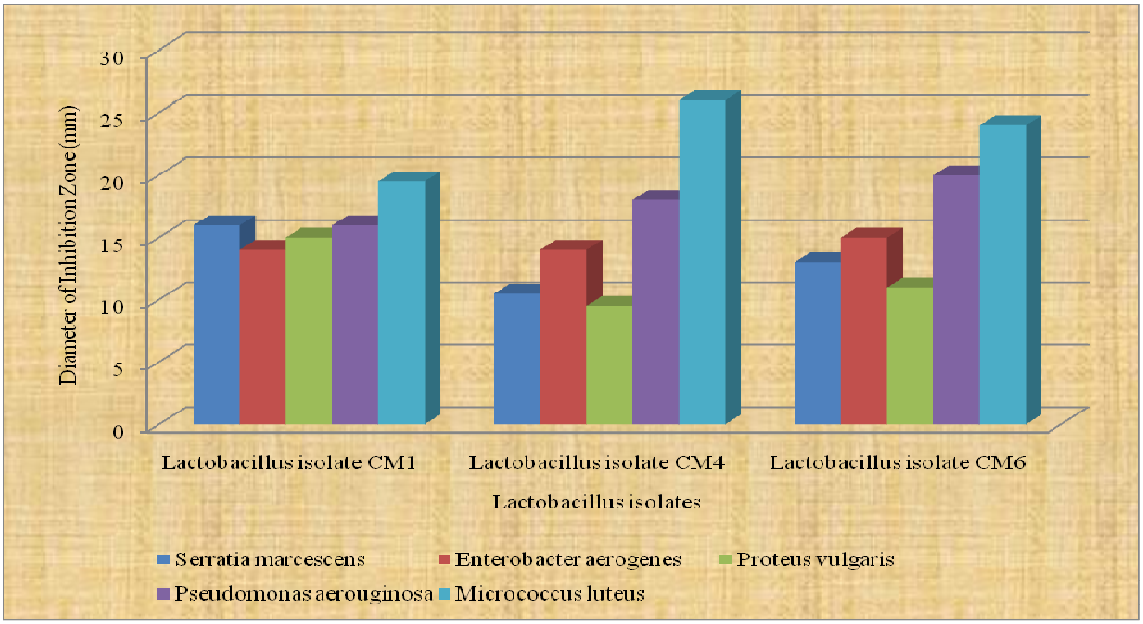

Figure-1
Antibacterial Activity of Lactobacillus Isolates against Different Pathogenic Bacteria (Without NaOH)

The bacterial supernatants with NaOH of all the three *Lactobacillus* isolates *Lactobacillus plantarum* CM1, *Lactobacillus fermentum* CM4 and *Lactobacillus casei* subsp. *casei* CM6 showed highest inhibition zones against *Micrococcus luteus* (table 2 and figure 2). The sizes of inhibition zones obtained for *Lactobacillus plantarum* CM1, *Lactobacillus fermentum* CM4 and *Lactobacillus casei* subsp. *casei* CM6 against *Micrococcus luteus* were 15.5, 21.0 and 14.0 mm respectively. For *Lactobacillus plantarum* CM1 the sizes of inhibition zones against rest of the indicator organisms were ranged from 10.0 to 12.0 mm. For *Lactobacillus fermentum* CM4 and *Lactobacillus casei* subsp. *casei* CM6 the sizes of inhibition zones against rest of the indicator organisms were ranged from 11.0 to 13.5 mm and 9.0 to 12.5 mm respectively.

Table-2
Antibacterial activities of *Lactobacillus* isolates against different pathogenic bacteria (With NaOH)

S. No.	Name of organism	Diameter of clear zone (mm) including well diameter					
		Serratia marcescens	***Enterobacter aerogenes***	***Proteus vulgaris***	***Pseudomonas aerouginosa***	***Micrococcus luteus***	**Mean**
1.	*Lactobacillus plantarum* CM1	10.5	11.5	10.0	12.0	15.5	11.9
2.	*Lactobacillus fermentum* CM4	12.0	11.0	13.5	11.0	21.0	13.7
3.	*Lactobacillus casei* subsp. *casei* CM6	11.5	10.5	09.0	12.5	14.0	11.5
4.	Mean	11.333	11.000	10.833	11.833	16.833	12.37

CRD ANOVA for Antibacterial activities of *Lactobacillus* isolates against different pathogenic bacteria (With NaOH)

S. No.	Source	DF	SS	MS	F	SE(m)	CD(5)	CD(1)
1.	A	2	0.274669	0.137335	27.466**	0.0224	0.0674	0.09325
2.	B	4	1.53134	0.382834	76.565**	0.02887	0.08702	0.1204
3.	A x B	8	0.548664	0.0685831	13.716**	0.05	0.1507	0.2085
4.	Error	15	0.0750017	0.00500011				

CV = 5.7179, A = *Lactobacillus* isolates, B = Indicator bacteria, *, ** Significant at 5 and 1 percent level of significance

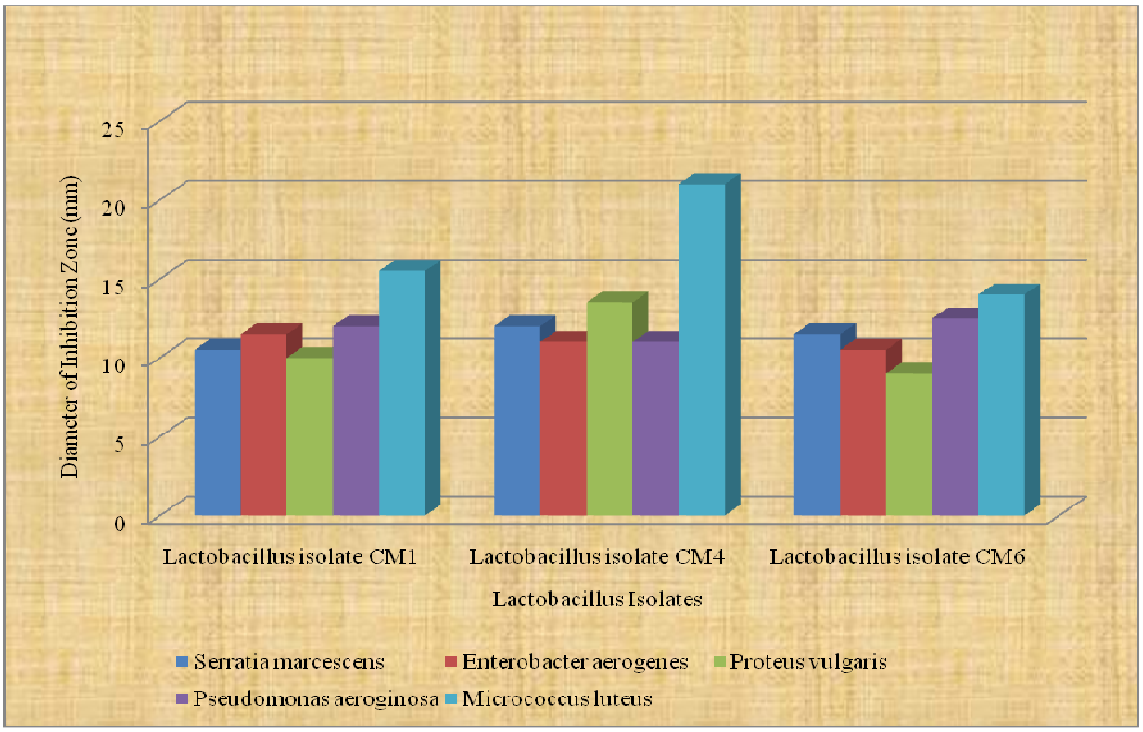

Figure-2
Antibacterial Activity of Lactobacillus Isolates against Different Pathogenic Bacteria (With NaOH)

Discussion: Antibacterial activity is one of the important properties for a strain to be a functional probiotic. The production of antimicrobial compounds by a probiotic organism provides a competitive edge to their survival and proliferation in GI tract and can also help in eradication of pathogenic bacteria. A variety of antimicrobial compounds such as organic acids, hydrogen peroxide, ethanol, acetaldehyde, acetoin, fatty acids, low molecular mass compounds and bacteriocins[6] are produced by lactobacilli.

In the present study both the bacterial supernatants normal supernatant and supernatant neutralized with NaOH of all the three *Lactobacillus* isolates showed the demonstrable antibacterial activities against both gram-positive and gram-negative pathogenic bacteria (table3.1 to 3.2). This observation showed stronger agreement with the report which reveals that *Lactobacillus* strains showed antibacterial activities against gram-positive as well as negative bacteria[7]. All the three *Lactobacillus* isolates showed broad inhibitory spectrum against both gram-positive and gram-negative bacteria[8,9].

The bacterial supernatant without NaOH of all the three strains showed stronger activity as compare to that of neutralized supernatant. Similar findings were also reported by researcher who proposed that the weak antibacterial activities in neutralized supernatant may be due to the other factors except acid and may be due to the production of bacteriocins that are active against pathogenic organisms at the optimum pH[10]. This may be the possible reason for antibacterial activities in neutralized supernatant in the present study.

Conclusion

As the antagonistic activity is one of the crucial property for a strain to be a probiotic. All the three *Lactobacillus* isolates showed demonstrable antibacterial activity. These *Lactobacillus* isolates could be used as potent probiotics. Further research related to the evaluation of these isolates for antibacterial compounds will be conducted.

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References

1. Shokryazdan P., Sieo C.C., Kalavathy R., Liang J.B., Alitheen N.B., Jahromi M.F. and HO W.Y., Probiotic potential of *Lactobacillus* strains with antimicrobial activity against some human pathogenic strains, *BioMed Res. Inter.,* 1-16 **(2014)**

2. Reid G., Jass J., Sebulsky M.T. and McCormick J.K., Potential uses of probiotics in clinical practice, *Clin. Microbiol. Rev.,* **16(4)**, 658-72 **(2003)**

3. Hassanzadazar H., Ehsani A., Mardani K. and Hesari J., Investigation of antibacterial, acid and bile tolerance properties of lactobacilli isolated from Koozeh cheese, *Vet. Res. Forum.,* **3(3)**, 181-85 **(2012)**

4. Puniya M., Sangu K.P.S., Bhardwaj A., Gupta D., Kumar S., Dhewa T. and Pant S., Probiotic and functional attributes of *Lactobacillus* spp. isolated from human faeces, *J. Res. Antimicrob.,* **1,** 32-42 **(2012)**

5. Schillinger U. and Lucke F.R., Antibacterial activity of *Lactobacillus sake* isolated from meat, *Appl. Enviro. Microbiol.,* **55**,1901-06 **(1989)**

6. Abdelbasset M. and Djamila K., Antimicrobial activity of autochthonous lactic acid bacteria isolated from Algerian traditional fermented milk "Raib", *African J. Biotechnol.,* **7(16)**, 2908-14 **(2008)**

7. Meira S.M.M., Helfer V.E., Velho R.V., Lopes F.C. and Brandelli A., Probiotic potential of *Lactobacillus* spp. isolated from Brazilian regional ovine cheese, *J. Dairy Res.,* **79**, 119-27 **(2012)**

8. De Waard R., Garssen J., Bokken G.C. and Vos J.G., Antagonisticactivity of *Lactobacillus casei* strain *shirota* against gastrointestinal *Listeria monocytogenes* infection in rats, *Int. J. Food Microbiol.,* **73**, 93-100 **(2002)**

9. Sirilun S., Chaiyasut C., Kantachote D. and Luxananil P., Characterisation of nonhuman origin probiotic *Lactobacillus plantarum* with cholesterol-lowering property, *AfricanJ. Microbiol. Res.,* **4(10),** 994-1000 **(2010)**