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Antimicrobial activity of *Lactobacillus* isolated from raw milk of Cow

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ABSTRACT

Altogether, 7 strains of *Lactobacillus* were isolated from raw milk of cow. Antimicrobial activity of isolated isolates was tested against three pathogenic bacteria: *E. coli*, *Proteus vulgaris, Staphylococcus aureus.* Out of 7 isolates, 3 belong to *L. acidophilus,* 2 to *L. delbrueckii* and 2 to *L. casei.* All isolates showed antimicrobial activity against all selected pathogens. Maximum inhibition zone was measured for isolate no. *PLA3* (*L. acidophilus*) against *Proteus vulgaris* (7.2 mm). Maximum inhibition against *E. coli* was recorded for isolate no. *PLA3* (6.8 mm) followed by *PLC1* (*L. casei*) (6.7 mm). Maximum inhibition against *Staphylococcus aureus* was recorded for *PLA3* (6.5 mm) followed by *PLD1* (*L. delbreuckii*) (6.3 mm).

Key Words - *Lactobacillus*, antimicrobial activity, pathogenic bacteria, isolates

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INTRODUCTION

Lactobacilli are gram positive, non-spore forming non-motile lactic acid bacteria found in milk, milk products, mammalian mucosal membrane, plant materials, sewage fermenting materials and spoiled food. *Lactobacilli* produce bacteriocin which inhibits the growth of pathogenic bacteria. Bacteriocin produced by different *Lactobacilli* differs in their type. Some of them inhibit the growth of gram negative bacteria, some other inhibit the growth of gram positive bacteria while some other are able to inhibit the growth of both gram positive and gram negative bacteria. Bacteriocin produced by *L. salivarius* subsp. *Salvarius* ucc118 can inhibit the growth of large number of pathogenic bacteria including *Listeria, Staphylococcus, Salmonella.* Similarly *Bacteriocin* produced by *Lactococcus lactis* inhibit the growth of *Clostridium*. Bacteriocins from some species of Lactobacilli are able to inhibit the growth of *Helicobacter pylori* (Gotteland *et al.* 2006). *Lactobacilli* are considered as probiotic. Probiotics are defined as living microbes which upon digestion exert health benefits beyond inherent basic nutrition (Tannock, 2002).

Probiotic microbes balances gut microflora and play important role in health maintenance (Schrezenmeir and Vrese, 2001). Probiotic bacteria protect against harmful microorganisms and strengthen host's immune system (Soccol, 2010; Pundir *et al*., 2013).

MATERIAL & METHODS

Milk samples were collected from villagers of Saharsa district who domesticated Cow. Milk samples were serially diluted up to 10^{-5} dilution and inoculated in MRS medium. Cultures were incubated at 37°C for 24 hours. Individual colony from MRS medium transferred to fresh MRS medium to get pure culture. Isolates were tested for gram staining, biochemical test, sugar fermentation test and identified on the basis of their characters using Standard monograph (Bergey's manual of Systematic Bacteriology, 2012).

Antimicrobial test:

For antimicrobial test of isolates three pathogenic bacteria *Escherichia coli, Proteus vulgaris* and *Staphylococcus aureus* were selected. Pathogenic bacteria were obtained from local pathological labs and cultured in Nutrient agar broth. Antimicrobial test of isolates were performed by disc diffusion method. Petri dish containing Muller Hinton agar was inoculated by Lawn culture method. On each culture plate filter paper disc soaked with cell free cultures of isolates were placed at the center. Cultures were incubated at 37°C for 24 hours and inhibition zone was measured for each isolate.

RESULT

Altogether, 7 isolates were isolated from milk samples out of which 3 belonged to *Lactobacillus acidophilus,* 2 belonged *L. delbrueckii* and 2 belonged to *L. casei*. Isolate codes are mentioned in Table 1.

Table 1: Species and their isolate codes

Species	Isolate code
L. acidophilus	PLA1, PLA2, PLA3
L. delbrueckii	PLD1, PLD2
L. casei	PLC1, PLC2

All isolates were gram positive, 'Rod shaped' forming 'Creamy white' colonies (Table 2), catalase, oxidase and indole test for all isolates were negative (Table 3). All isolates were glucose, lactose, mannitol and raffinose positive while fructose, sucrose and arabinose negative (Table 4).

Table 2: Cell and Colony morphology of isolates

Isolates	Cell Shape	Colony morphology	Gram Staining
PLA1	Rod shaped	Creamy white	+ve
PLA2	Rod shaped	Creamy white	+ve
PLA3	Rod shaped	Creamy white	+ve
PLD1	Rod shaped	Creamy white	+ve
PLD2	Rod shaped	Creamy white	+ve
PLC1	Rod shaped	Creamy white	+ve
PLC2	Rod shaped	Creamy white	+ve

Table 3: Biochemical test of isolates

Isolates	Catalase	Oxidase	Indole
PLA1	-ve	-ve	-ve
PLA2	-ve	-ve	-ve
PLA3	-ve	-ve	-ve
PLD1	-ve	-ve	-ve
PLD2	-ve	-ve	-ve
PLC1	-ve	-ve	-ve
PLC2	-ve	-ve	-ve

Table 4: Sugar fermentation test of isolates

Isolates	Glu	Lact	Fruc	Sucr	Mann	Raff	Arab
PLA1	+ve	+ve	-ve	-ve	+ve	+ve	-ve
PLA2	+ve	+ve	-ve	-ve	+ve	+ve	-ve
PLA3	+ve	+ve	-ve	-ve	+ve	+ve	-ve
PLD1	+ve	+ve	-ve	-ve	+ve	+ve	-ve
PLD2	+ve	+ve	-ve	-ve	+ve	+ve	-ve
PLC1	+ve	+ve	-ve	-ve	+ve	+ve	-ve
PLC2	+ve	+ve	-ve	-ve	+ve	+ve	-ve

Antimicrobial test:

All isolates showed antimicrobial activity against all selected pathogens. Maximum inhibition zone was measured for isolate no. *PLA3* against *Proteus vulgaris* (7.2mm). Maximum inhibition against *E. coli* was recorded for isolate no. *PLA3* (6.8mm) followed by *PLC1* (6.7mm). Maximum inhibition against *Staphylococcus aureus* was recorded for *PLA3* (6.5mm) followed by *PLD1* (6.3mm). The result is shown in Table 5.

Table 5: Inhibition zone of Pathogensdes

Isolate	Inhibition zone (mm) of Pathogens		
	E. coli	*Proteus*	*Staphylococcus*
PLA1	5.5	5.5	4.7
PLA2	5.8	6.3	5.2
PLA3	6.8	7.2	6.5
PLD1	6.2	6.8	6.3
PLD2	6.5	5.9	5.5
PLC1	6.7	6.8	5.7
PLC2	5.6	5.2	4.5

CONCLUSION

Antimicrobial activity of *Lactobacillus* isolates were tested against three pathogenic bacteria *E. coli, Proteus vulgaris* and *Staphylococcus aureus*. Altogether, 7 isolates belonging to *L. acidophilus, L. delbrueckii* and *L. casei* were isolated from raw milk of cow and tested against pathogenic bacteria. Isolates were assigned code as *PLA1, PLA2, PLA3* for isolates of *L. acidophilus*, *PLD1, PLD2* for isolates of *L. delbrueckii* and *PLC1, PLC2* for isolates *L. casei*.

All isolates showed antimicrobial activity against all selected pathogens. Maximum inhibition zone was measured for isolate no. *PLA3* against *Proteus vulgaris* (7.2mm). Maximum inhibition against *E. coli* was recorded for isolate no. *PLA3* (6.8mm) followed by *PLC1* (6.7mm). Maximum inhibition against *Staphylococcus aureus* was recorded for *PLA3* (6.5mm) followed by *PLD1* (6.3mm).

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