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Antagonistic activity of *Lactobacilli* derived from raw milk

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Abstract- Altogether 15 milk samples were collected from local dairy firm of Madhepura district and *Lactobacilli* were isolated. Altogether 3 isolates were obtained, LM1, LM2 and LM3 which were identified as *Lactobacillus casei*, *Lactobacillus brevis* and *Lactobacillus fermentum* respectively. The identification were made on the basis of their morphological, biochemical and sugar test as prescribed in Bergey's manual of systematic Bacteriology (second edition-2001). The antagonistic study of isolated strain was made against four pathogenic bacteria *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus* and *Proteus sp.*. The inhibition zone against *Pseudomonas aeruginosa, Escherichia coli* and *Proteus sp.*was observed maximum by *Lactobacillus brevis* and against *Staphylococcus aureus*, the maximum inhibition zone was observed by *Lactobacillus fermentum*.

Key words: Niche, Niche Relaxation, Correlation, Amoeba, Copepod

INTRODUCTION

Raw milk is natural growth medium for micro-organism. An integral part of raw milk microflora is species of *Lactobacilli* as *Lactobacillus casei*, *L. plantarum*, *L. rhamnosus*, *L. curvatus*, *L. bravis*, *L. fermentum* and some other Lactic acid bacteria like *Leuconostoc lactis*.

Lactobacilli are rod shaped gram positive, Catalase negative, non-pathogenic bacteria present in milk and milk products. Several *Lactobacillus* strains are used as starter culture for the manufacture of fermented food.[1] These bacteria play a vital role in our daily life for fermentation, preservation, production and of wholesome food.

Lactic acid bacteria produced various compounds as organic acids, diacetyls, hydrogen peroxide, Vitamins and anti-microbial substance, bacteriocin. Antimicrobial activity of *Lactobacillus* against *E. coli*, *S. aureus* and *Solmonella* was reported by several workers.[2-5]

Lactobacillus casei have a wide range of pH and temperature and complement the growth of *L. acidophillus*, a producer of enzyme amylase. *L. sporogenesis* supports the growth of beneficial bacteria and help to maintain a healthy balance of microflora in the intestinal environment. A lot of LAB has been noted to have nutritional benefits, improve Lactose utilization, and have anti-cholesterol, anti-carcinogenic activity. Intestinal Lactic acid bacteria are considered to have health benefits for human being and introduced as probiotic including *L. rhamnosus*, *L. casei* and *L. johnsonii*.[6]

MATERIAL & METHOD

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Milk samples were collected from local diary firm of Madhepura in 500ml sterilized bottles and brought to the laboratory in ice boxes. From each sample, 1ml of milk was mixed in 10ml distilled water and serial dilution was made up to 10^{-5}. From each sample, 0.1ml inoculum was inoculated in MRS medium and incubated at 30°C temperature for 48 hours. Colonies were purified by sub-culturing in same medium. From each plate, colonies were examined for morphological study, gram staining, biochemical test and sugar test. On the basis of biochemical test and sugar test, isolates were categorised in following three categories: LM1, LM2 and LM3. The strains were identified on the basis of their morphological character, biochemical and sugar test as prescribed in Bergey's manual of systematic Bacteriology (second edition-2001).

Four pathogens *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus* and *Proteus sp.*were obtained from local pathological lab. All test pathogenic bacteria were cultured in Nutrient broth.

Antagonistic study:

For the antagonistic study of isolated strains of *Lactobacilli* against pathogenic bacteria was performed by well diffusion method.

Petri dishes containing 20ml of Muller Hinton agar were prepared and inoculated with 0.1ml pathogenic culture. The plates were allowed to solidify and stored in refrigerator for 2 hours. Four wells were made in culture plate and filled using different concentration (0.01ml, 0.02ml, 0.03ml and 0.04ml) of cell free filtrate of each isolate. Plates were incubated at 37°C for 24 hours. The diameter of inhibition zone was measured using Callipers.

Table 1- Biochemical test

Sl. No.	Isolate	Gram staining	Indole	MR	VP	Catalase	Oxidase	Nitrate reducation
1	LM1	+ve	-ve	-ve	-ve	-ve	-ve	+ve
2	LM2	+ve	-ve	-ve	-ve	-ve	-ve	+ve
3	LM3	+ve	-ve	-ve	-ve	-ve	-ve	+ve

Table 3- Antagonistic activity of selected isolates against pathogens

Sl. No.	Pathogens	LM1	LM2	LM3
1	*Pseudomonas aeruginosa*	7mm	13mm	6.5mm
2	*Escherichia coli*	7.5mm	13.5mm	9.5mm
3	*Staphylococcus aureus*	9.5mm	10mm	12.5mm
4	*Proteus sp.*	8mm	14mm	11mm

RESULT

Altogether 15 samples were tested in which 3 isolates were obtained which were assigned as LM1, LM2 and LM3. All isolates were gram positive while Indole, Catalase and Oxidase show negative. In sugar test, LM1 was negative in Glucose and sorbitol and positive in all other tested sugars. LM2 was positive for Xylose and negative for all other sugars. LM3 was negative in Rhamonose and positive in all other test sugars. On the basis of biochemical test and sugar fermentation test, LM1 was identified as *Lactobacillus casei*, LM2 as *Lactobacillus brevis* and LM3 as *Lactobacillus fermentum*.

The result is tabulated in Table no. 01 and 02. All three isolates strains inhibited the growth of all four pathogenic bacteria under test. The inhibition zone varies for different strains for different pathogenic bacteria. The maximum inhibition zone against *Pseudomonas aeruginosa* was of LM2 (13mm) and minimum of LM3 (6.5mm), against *E. coli* the highest inhibition zone was recorded for LM2 (13.5mm) and minimum for LM1 (7.5mm). The maximum inhibition zone against *S. aureus* was recorded for LM3 (12.5mm) and minimum for LM1 (9.5mm) and Inhibition zone against *Proteus* was maximum for LM2 (14mm) and minimum for LM1 (8mm). The result is tabulated in Table no. 03.

CONCLUSION

Altogether 15 milk samples were collected from local area of Madhepura district. *Lactobacilli* from each milk were isolated by culturing in MRS medium, Gram staining, Biochemical test and morphological study of bacteria from

Table 2- Sugar fermentation test

Sugar test	LM1	LM2	LM3
Xylose	+ve	+ve	+ve
Cellubiose	+ve	-ve	+ve
Glucose	-ve	-ve	+ve
Mannitol	+ve	-ve	+ve
Ramonose	+ve	-ve	-ve
Raffinose	+ve	-ve	+ve
Ribose	+ve	-ve	+ve
Sorbitol	-ve	-ve	+ve
Trehalose	+ve	-ve	-ve
Fructose	+ve	-ve	+ve

all samples were performed. Three different isolates were obtained from all samples. On the basis of their biochemical study and sugar test, the isolates were identified as *Lactobacillus casei*, *Lactobacillus brevis* and *Lactobacillus fermentum*. The antagonistic study of these *Lactobacilli* species were performed against four pathogenic bacteria- *Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus* and *Proteus* sp.. The antagonistic study was performed by well diffusion method. The inhibition zone against *Pseudomonas aeruginosa, Escherichia coli* and *Proteus sp.* was observed maximum by *Lactobacillus brevis* and against *Staphylococcus aureus*, the maximum inhibition zone was observed by *Lactobacillus fermentum*.

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