Research Article

EVALUATION OF PROBIOTIC POTENTIAL OF *LACTOBACILLUS FERMENTUM* STRAIN

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ABSTRACT

In present study, milk samples were collected from different regions of Jodhpur. *Lactobacillus* strains were isolated on MRS agar medium. Several biochemical and physiological tests were performed for identification of these isolates. The isolates were screened for their antimicrobial activity against some human pathogenic bacteria by using agar well diffusion method. One of the isolates with significant antagonistic property against some Gram positive and Gram negative human pathogens was found to be *Lactobacillus fermentum*. The 16s rRNA sequence of this isolate was done and the sequence was submitted in the NCBI Genbank database. From all the results it is concluded that the isolate was *L. fermentum* with significant probiotic potential.

Keywords: *Lactobacillus fermentum*, antimicrobial activity, probiotic, bacteriocins.

INTRODUCTION

Lactic acid bacteria (LAB) are a group of bacteria which are heterogeneous and possess diverse type of properties. They are characterized as Gram positive, usually non motile, non sporulating bacteria that produce lactic acid as a major or sole product of fermentative metabolism. *Lactobacillus* is one of the important genus of LAB group. It plays major role in dairy industry due to its fermenting ability. Besides their role in dairy industry, lactobacilli are getting importance as a source of probiotics due to their antimicrobial property[1]. The aim of the study was to isolate the strains of *Lactobacillus* from milk samples and to determine their antimicrobial property against some human pathogenic bacteria.

MATERIALS AND METHODS

Sample collection: Milk samples were collected from various regions of Jodhpur, in sterile screw-cap bottles and brought to the laboratory. In the laboratory, enrichment process was carried out by adding 10 ml of sample to 80 ml of MRS broth (Himedia) in conical flask. The enriched samples were incubated at 37°C for 24 hours. The enrichment process was conducted in triplicates.

Isolation: The isolation process was carried out by streaking the enriched samples on MRS agar (Himedia) plates[2] and incubating the plates at 37°C for 24 hours. Colonies with typical characteristics were randomly selected from the plates and tested for cell morphology, Grams staining and catalase test before further biochemical tests. The bacterial cultures which were Gram positive, rod shaped and catalase negative were further sub cultured to get pure isolates. These isolates were stored and maintained in litmus milk for further studies[3].

Characterization and identification

The identification of the isolates was done by morphological, biochemical and molecular methods. The biochemical characterization included Gram's staining, catalase test, glucose fermentation test, litmus milk coagulation, carbohydrates fermentation reaction, Voges-Proskauer test, Arginine hydrolysis test and growth at 15°C and 45°C. The carbohydrates tested were arabinose, cellobiose, fructose, galactose, lactose, maltose, mannitol, raffinose, ribose, rhamnose, sucrose, xylose and trehalose. Glucose and sterile water were used as positive and negative control respectively[4].

Molecular characterization of the isolates: The 16s rRNA sequencing was done by isolating and purifying the genomic DNA of the isolates. The 16s rRNA fragment was amplified using universal primers (forward) i.e. 518F (seq. CCAGCAGCCGCGGTAATACG) and (reverse) i.e. 800R (seq. TACCAGGGTATCTAATCC)[5]. The obtained sequences were analyzed for homology using BLAST-N.

Antibacterial activity of the *Lactobacillus* isolates:

The antibacterial activity of the isolated *Lactobacillus* strains was determined against some clinical isolates viz. *Escherichia coli, Staphylococcus aureus*, *Klebsiella pneumonia* and *Bacillus subtilis* by agar well diffusion method [6]. The test bacteria (clinical isolates) were obtained from a private Diagnostic Centre of Jodhpur.

The test bacteria were inoculated in nutrient broth and incubated at 37°C for 24 hours. Approximately 10^7cfu/ml (i.e. heavy growth) of the bacteria to be tested for sensitivity were inoculated into 20 ml of nutrient agar and poured in the petridish. For the detection of the antibacterial activity of the *Lactobacillus* isolates, these isolates were inoculated in MRS broth containing only 0.2% glucose (MRS-0.2) and incubated at 37°C for 24 hours[7]. After incubation, the broth was centrifuged at 6000Xg for 15 min. and the supernatant was filtered

through a 0.2 µm pore size cellulose acetate filter paper. The pH of the filtrate was adjusted to 7 by 1N NaOH. The cell free solution thus obtained was used for detection of antibacterial activity against pathogenic bacteria in the inoculated nutrient agar. 100 µl of the supernatant was filled in 8mm diameter agar well cut in the inoculated nutrient agar plate. The inoculated plates were incubated for 24 hrs at 37°C and the diameter of the inhibition zone was measured in millimeter.

RESULTS AND DISCUSSION

30 strains of *Lactobacillus* were obtained from milk samples. All the strains were rod shaped, Gram positive, catalase negative and oxidase negative. One of the strains showing significant antagonistic activity was found to be *L. fermentum* on the basis of certain biochemical characteristics as shown in table 1. The identification was further confirmed by 16s rRNA gene sequencing. A BLAST N search for 1488 bp 16s rRNA gene sequence of the isolate showed 99% similarity with *Lactobacillus fermentum.*

Table 1: The biochemical and physiological characteristics of *L. fermentum* SRJ-23

Tests	Results
Gram's staining	+
Colony color	Pale yellow
Enzymatic activity:	
Catalase	-
Oxidase	-
Gas production from glucose	+
Indole formation	-
MR –VP test	-
Arginine hydrolysis test	+
Temperature for growth	
Optimum	37°C
Range	20-45°C
Conc. of NaCl (%w/v)	
1%	+
2%	+
3%	+
5%	-
Sugar fermentation test	
D-glucose	+
Sucrose	+
D-xylose	+
D-galactose	+
Maltose	+
L-arabinose	-
Lactose	+
Raffinose	+
Rhamnose	+
Fructose	+
Melibiose	-
Sorbitol	+
Mannitol	-
Mannose	-
Glycerol	-
Chemical characteristics	
G+C content (mol %)	53.12 %
(+)=positive and (-)= negative	

Nucleotide sequence accession number: The nucleotide sequence of 16s rRNA from the isolate investigated in this study has been deposited in the NCBI GenBank database library under Accession no. JN798180 and as *L. fermentum* SRJ-23.

The antagonistic effect of the *L .fermentum* strain is presented in table 2. All the test pathogens were significantly inhibited by *L.* fermentum strain. *Bacillus subtilis* was most inhibited with a zone of inhibition of 22 mm while *Klebsiella pneumoniae* was least inhibited with a zone of 9 mm. Even though the inhibition of *K. pneumoniae* was significant. It is clearly observed that the *L. fermentum* strain is showing antagonistic activity against both Gram positive and Gram negative bacteria. The antimicrobial effect exerted by *Lactobacillus* is mainly due to production of lactic acid, acetic acid, diacetyl, H_2O_2, bacteriocins, acetoin or other compounds[8, 9]. The production of these compounds depends on the strain, media and its composition and other physical parameters[10].

Table 2: Antibacterial activity of *Lactobacillus fermentum*

Clinical Isolates	Zone of Inhibition (mm)	
	L. fermentum	**Positive Control**
S. aureus	20.0±0.56	12.0±0.57
B. subtilis	22.0±0.57	19.0±0.58
E.coli	15.5±0.58	11.0±0.57
K.pneumoniae	9.0±0.58	11.8±1.0

CONCLUSION

The *L. fermentum* strain SRJ-23 had shown significant antimicrobial activity against various clinical pathogens. The observed antimicrobial activity was not due to acidic pH because the supernatant was maintained at a neutral pH. This suggests that acid is not the cause of bactericidal activity in this case. The inhibition could be due to the production of proteinaceous substance like bacteriocin which is characteristic feature of many lactobacilli. Bacteriocins are important in biopreservation of food as well as probiotic compounds. Thus we can conclude that this *L. frementum* strain could be a promising source of probiotics.

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