World Journal of Pharmaceutical and Life Sciences
WJPLS
www.wjpls.org
SJIF Impact Factor: 6.129

ANTAGONISTIC EFFECT OF LACTOBACILLUS SPECIES ISOLATED FROM DAIRY PRODUCTS AND FERMENTED FOOD ON INTESTINAL PATHOGENIC BACTERIAL SPECIES

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Article Received on 23/07/2020 **Article Revised on 13/08/2020** **Article Accepted on 03/09/2020**

ABSTRACT

The probiotic microorganisms especially Lactic acid bacteria (LAB) are reported to produce many metabolites including organic acids, hydrogen peroxide, diacetyl and bacteriocins; that could be utilized in vast number of applications. Besides the use of probiotic organism in variety of industrial food and feed fermentations, they possess strong antagonistic properties towards other related microorganisms, including food spoilage organisms and pathogens. Since microbial metabolites derived from probiotics have shown the inhibitory activity against the many pathogens including E.coli, S. aureus, S. typhi, S. dysenteriae, B. anthracis etc. In present investigation also different types of fermentating food stuffs and dairy products were used to isolate probiotic *Lactobacilli* with antimicrobial potential which probably may secret antimicrobial proteinaceus substances which are collectively called as bacteriocins. The pure culture of indigenously isolated *Lactobacilli* were inoculated in sterile MRS broth and incubated at 37°C for 48 hours. The *Lactobacillus* isolates were cultured in the volume of 5 ml in test tube which were subjected to centrifugation at 10,000 RPM in a high speed centrifuge for 15 minutes at ambient temperature after the period of incubation when growth of microbes were observed in the form of turbidity. The procedure followed was with reference to the procedures suggested. Well diffusion method was adapted to check the antimicrobial potential of indigenously isolated Lactobacilli from different fermenting food and dairy products Out of the 14 indigenously isolated *Lactobacillus* spp. the isolates which were observed to impart inhibitory effect at a concentration of 20 µl of fermentation extract in each well of media plates are LB-2, LB-5, LB-6, LB-7, LB-11, LB-12, LB-13 and LB-14 towards test microbial species used in present work.

KEYWORDS; Lactobacillus, Pathogens, Fermentation Antagonistic properties.

INTRODUCTION

Several types of proteinaceous compound such as nisin, acidophillin, bulgaricin and diplococcin secreted by probiotic bacteria with antimicrobial properties which are collectively called as Bacteriocins. These bacteriocins are protein compounds with growth inhibition ability of sensitive pathogenic bacteria and different degradation system in digestive system compared with antibiotics.[1-2] Thus the present investigation was intended to focuses on the use of proteinaceous antimicrobial substances secreted by indigenous probiotic bacteria against intestinal pathogenic micro-organism. Nowadays, probiotics are used not only as a driver of growth but also as a stimulator of the immune system and prevention of many diseases.[3] Nonetheless, there are huge technological difficulties for probiotics which, being of intestinal origin, are delicate to numerous environmental hassles. Microbes are able to produce metabolites which are generally of secondary origin usually late in the growth cycle (idiophase). These metabolites with enormous range of biological activities find its use and applications in pharmaceutical and cosmetics, food, agriculture, farming etc. These include compounds with anti-inflammatory, hypotensive, anti-tumor, anti-cholesterolemic activities, and also insecticides, plant growth regulators and environmental friendly herbicides and pesticides The probiotic microorganisms are generally employed in dairy products in general in order to have the therapeutic benefits of probiotic functional food.[4] The present investigation involves the isolation of Lactobacillus spp. from dairy and other food products and the effect of their fermentation products on intestinal pathogenic bacterial spp.

MATERIALS AND METHODS

Sample collection

The samples for isolation of LAB were included as raw milk, fermented milk, curd, buttermilk, cottage cheese, khoa, fermenting dough, dhokla batters, Dosa batter, rotting fish, rotting food etc.

Isolation of *Lactobacillus* spp. and Identification

Various samples collected from different resources were first homogenized followed by their serial dilution in sterile distilled water under aseptic condition in laboratory. Since for the isolation of any *Lactobacillus* species or stain, mostly MRS agar media is used as a selective medium (deManRogosa and Sharpe). The dilutions from 10^{-5} to 10^{-7} were used out of the dilution series to inoculated the culture medium by spread plated method followed by its incubation at 37°C for 48 to 72 hours.

Test microbial strains

The antimicrobial activity of the indigenously isolated *Lactobacillus* spp. from different sources was done on each of the Gram positive and Gram negative indicator intestinal pathogenic bacteria. The MTCC cultures of Gram + ve St*aphylococcus aureus* and Gram –ve *Escherichia coli* were used for this experiment which was kindly provided by IMTech Chandigarh, India.

Preparing supernatant of *Lactobacillus* spp.

The pure culture of indigenously isolated *Lactobacilli* were inoculated in sterile MRS broth (HiMedia) and incubated at 37°C for 48 hours. Composition of MRS broth is same as that of the MRS media excluding agar powder. The *Lactobacillus* isolates were cultured in the volume of 5 ml in test tube which were subjected to centrifugation at 10,000 RPM in a high speed centrifuge for 15 minutes at ambient temperature after the period of incubation when growth of microbes were observed in the form of turbidity.

Antimicrobial Activity by Well Diffusion Method

Well diffusion method was adapted to check the antimicrobial potential of indigenously isolated Lactobacilli from different fermenting food and dairy products .The nutrient agar plates were prepared and the 48 hours old culture of test microbial species was inoculated on each plate separately with *E. Coli* (MTCC-1687) and *S. aureus* (MTCC- 737). The 5 well on each plate were punched of 6 mm diameter using wide portion of a sterile microtip. After labelling the wells on back side of the plate each well is filled with 20 µl of culture supernatant prepared from the *Lactobacillus* cultures. The plates were incubated at 37^{o}C for 24 hours and then examined for clear zones of inhibition due to the supernatant or extracts of Lactobacillus cultures.

RESULTS AND DISCUSSION

Isolation of *Lactobacillus* spp

In the beginning out of 79 master culture plates prepared by utilizing 12 different types of samples there were 21 bacterial isolates were pure cultures coming from all the 12 sample types taken under study. The bacterial isolates were further sorted and reduced to a final number of 14 pure cultures on the basis of resource from samples multiple times and morphological similarities which included the shape, size and colour of bacterial colony. The growth on selective media, colony morphology, microscopy and biochemical confirms that the isolated bacterial isolates were of Lactobacillus species.[5] These 14 *Lactobacillus* spp. isolates were designated with new codes as LB_1, LB_2, LB_3,…… $LB_{14}$and were used in further studies.

Screening of *Lactobacillus* spp. with Antimicrobial Potential

Out of the 14 indigenously isolated *Lactobacillus* spp. the isolates which were observed to impart inhibitory effect at a concentration of 20 µl of fermentation extract in each well of media plates are LB-2, LB-5, LB-6, LB-7, LB-11, LB-12, LB-13 and LB-14 towards test microbial species used in present work. These 8 bacterial isolates with the codes just mentioned does not have the similar effects in all the cases. Only two LAB isolates with codes LB-2and LB-7 out of all 8 LAB isolates showed inhibition towards both the test microbial strains.The bacterial isolates LB-5, LB-11, LB-12 and LB-14 were reported with inhibitory activity against test *E. coli* (MTCC-1687) only whereas the other isolates with codes LB-6 and LB-13 were observed to be positive for inhibitory activity against the tests S. aureus (MTCC-737).

The fermentation extract prepared in liquid MRS medium responded for antimicrobial activity in certain number of cultures against the test microbial species as depicted in table 2. These antagonistic substances against the pathogenic microbial species would have been the great efforts of scientists if it comes from natural origin instead of synthetic and harmful drugs.[6-8] The antimicrobial activity in the fermentation extract was due to the secretion of some unknown chemical compounds because of fermentation activity of indigenously isolated *Lactobacillus* spp. from dairy and fermentating food articles.

Table 1: Antimicrobial potential of the fermentation extract of indigenous Lactobacillus isolates.

S.N	Isolates	Isolate Source	Antimicrobial potential against	
			E. coli (MTCC-1687)	*S. aureus* (MTCC-737)
1.	*LB-1*	Curd	–ve	–ve
2.	*LB-2*	Butter milk	+ve	+ve
3.	*LB-3*	Fermentating Milk	–ve	–ve
4.	*LB-4*	Curd	–ve	–ve
5.	*LB-5*	Fermenting dough	+ve	–ve
6.	*LB-6*	Vegetable waste	–ve	+ve
7.	*LB-7*	Rotting fish	+ve	+ve
8.	*LB-8*	Cottage cheese	–ve	–ve
9.	*LB-9*	Raw milk	–ve	–ve
10.	*LB-10*	Dhokla batters	–ve	–ve
11.	*LB-11*	Fermenting dough	+ve	–ve
12.	*LB-12*	Khoa	+ve	–ve
13.	*LB-13*	Curd	–ve	+ve
14.	*LB-14*	Cottage cheese	+ve	–ve

Table 2: Grouping of indigenous Lactobacillus isolates for inhibitory response.

S.N.	Group	Inhibitory Response *E. coli* and *S. Aureus*	Isolates included
1.	*Grp – 1*	*E. coli* only	LB-5, LB-11, LB-12, LB-14
2.	*Grp – 2*	*S. aureus* only	LB-6 and LB-13
3.	*Grp – 3*	*E. coli* and *S. aureus*	LB-2 and LB-7
4.	*Grp – 4*	no inhibition	LB-1, LB-3, LB-4, LB-8, LB-9, LB-10

The present investigation undertaken was targeted to find out the isolates of *Lactobacillus* species with antimicrobial proteins or peptides collectively referred to as bacteriocins that is why out of 79 initial master cultures from 12 different type of sample stuffs only 14 different *Lactobacillus* isolates were obtained in present study that were found positive in antimicrobial susceptibility against test microbial *E. coli* (MTCC-1687) and *S. aureus* (MTCC- 737) used for this study. Though all the 14 *Lactobacillus* isolates were microscopically, morphologically and biochemically characterized but only 4 isolates whose antimicrobial potential towards the test pathogen bacteria was affected by protease enzymes were considered for further investigation because their antimicrobial activity was observed to be due to the proteinaceus molecules secreted by then in fermentation liquor. These proteinaceus antimicrobial compounds producing bacteria can be used as natural preservatives,[9] Lactobacilli are Gram-positive and catalase-negative bacteria that are known as most important probiotics and desirable gut microflora.[10]

CONCLUSION

On the basis of experimental outcomes with reference to the objectives of present investigation undertaken, we can conclude that the variety of dairy and other food samples are rich in number of bacterial species including lactobacillus species where all bacteria of lactobacillus spp. do not show antimicrobial activity towards the test pathogenic bacterial strains. For many years, dairy products have been recognized as valuable products to human health,[11] In recent years, many scientists have isolated and identified LAB and lactobacilli from traditional products worldwide and have evaluated their antagonistic effects against various pathogens.[12] The antimicrobial potential of indigenous lactobacillus spp. against the bacterial species was due to either secretion of organic acids or proteinaceus compounds.

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