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Screening of *in vitro* Antimicrobial Potential of Fermentation Extracts Prepared from Indigenous *Lactobacillus* Isolates

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ABSTRACT

Recently, efforts are being made to efficiently employ the probiotic bacteria in non-dairy products either as supplemental additive or for fermentation which might have several advantages over the probiotic dairy products such avoiding milk protein allergies, lactose intolerance or hyper cholesterolemia. So the present study aimed at screening of certain indigenous lactobacillus species responsible with antimicrobial potential against common pathogenic microorganism when allowed to ferment in liquid culture medium. The indigenous Lactobacillus spp. were isolated using different dairy samples like milk, curd, butter milk, cheese etc., on selective MRS agar medium and pure cultures were partially identified referring Bergey's Manual of Systematic Bacteriology based on morphological, cultural and biochemical reactions. The selected pure cultures were allowed to ferment in MRS broth for 7 days at 37°C in with intermittent shaking of culture flasks. There were total 14 isolates of Lactobacillus spp. were obtained. According to the results of biochemical tests as described in table 1 the probability of LAB would have been *L.acidophilus, L.fermentum, L.plantarum, L.casei, L. ruteri* or *L.rhamnosus*. Out of the 14 indigenously isolated Lactobacillus spp., the cell free fermentation extract of the isolates LB-2, LB-3, LB-5, LB-6, LB-7, LB-9, LB-11, LB-12, LB-13 and LB-14 was found imparted antimicrobial activity over the test microbial species including *Escherichia coli* (MTCC-1687), *Pseudomonas aeroginosa* (MTCC-*1934), *Staphylococcus aureus* (MTCC-737) and *Candida albicans* (MTCC-227), due to unknown substances secreted within fermentation extract. The ability to produce antagonistic substances during the process of fermentation could be explored extensively for identification, purification and use in food preservation at both domestic as well as industrial levels.

Keywords: Therapeutics, Antimicrobial activity, Lactobacillus, Fermentation extracts

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INTRODUCTION

Changes are being observed in Indian dairy industry which is largest producer of milk in the world. Yoghurt or curd like dairy products are known more for their therapeutic significance than nutritional value which are formed due to the activity of certain mesophilic nonpathogenic microorganism naturally of added intentionally leads to fermentation of lactose for form lactic acid.It has a limited keeping quality of 1 - 2 days at ambient temperature and its quality is not retained for more than 1 week under refrigerated conditions[1,2]. Probiotic strains of Lactobacillus, *Bifido bacterium* and Saccharomyces are considered GRAS (Generally Recognized as Safe), where Genus Lactobacillus have been extensively investigated that includes genetically and physiologically diverse variety of bacteria which are characterized Gram- positive, non-sporing, forming, cocci or rod shaped, Catalase-negative and fastidious organisms that have been used as starter culture for fermentation of a wide range of food production including milk and milk products[3-5]. Probiotic microorganism are generally employed in dairy products like flavored milks, yogurts, icecreams, cheese etc., in general in order to have the therapeutic benefits of probiotic functional food[6]. But in recent days, efforts are being made to efficiently employ the probiotic bacteria in non-dairy products either as supplemental additive or for fermentation which might have several advantages over the probiotic dairy products such avoiding milk protein allergies, lactose intolerance or hyper cholesterolemia. The present investigation involved the screening of certain indigenous lactobacillus species responsible with antimicrobial potential against common pathogenic microorganism when allowed to ferment in liquid culture medium.

MATERIALS & METHODS

Sample Collection

Several dairy samples like milk, curd, butter milk, cheese etc., were collected from local resources in order to screen out the microbial species of genus Lactobacilli.

Bacterial Isolation and Identification

The collected samples were subjected to serial dilution within sterile distilled water under aseptic conditions. The dilutions of 10^{-5} to 10^{-7} were used to inoculate the culture media plates containing MRS agar media (de-Mann, Rogosa and Sharpe) (HiMedia India Pvt Ldt) which allows the selective growth of Lactic Acid Bacteria. The culture plates were incubated at 37°C for 48 hours in bacteriological incubator. The developed bacterial colonies after incubation were picked up and subculture on fresh MRS agar medium and nutrient agar medium to get the pure culture followed by their characterization by morphological, cultural andbiochemical reactions for partial identification referred in Bergey's Manual of Systematic Bacteriology [7,8].

Preparation of Fermentation

The pure culture of unknown lactobacillus isolates were subjected of fermentation in MRS liquid medium of which small amount of inoculum from bacterial colony was inoculated first in 10 ml broth which was scaled up to 50 ml after 48 hour. The fermentation was allowed for 7 days of incubation at 37°C in an incubator with intermittent shaking of culture flasks.

Antimicrobial Activity of Fermentation Extract

The culture broth containing unknown lactobacilli after incubation were first taken in 15 ml sterile tube and centrifuged at 5000 RPM to get the cell free fermentation extract. The supernatant was passed through 0.22µ sterile dissociable syringe filters with nitrocellulose membrane (Moxcare) and contained in fresh sterile tube. This filtration sterilized supernatant as fermentation extracts were used for antimicrobial activity against the test pathogenic microbial strains including *Escherichia coli* (MTCC-1687), *Pseudomonas aeroginosa* (MTCC-*1934), *Staphylococcus aureus* (MTCC-737) and *Candida albicans* (MTCC-227). The antimicrobial activity of the fermentation extract was determined by well diffusion method against the test pathogenic microbial species.

RESULTS AND DISCUSSION

Isolation and Identification of Lactobacillus spp

There were total 14 bacterial isolates obtained from difference dairy products. These isolates were designated as LB-1, LB-2, LB-3 upto LB-14. The pure cultures of these isolates were prepared from the creamy while pin point to large convex type of colonies developed on MRS agar media. The selected representative colonies were picked to prepare the pure cultures and were characterized as Gram positive, non-sporing rod shaped bacterium on the basis of staining and microscopic studies. The results of tests used for characterization of bacterial isolates are depicted in table 1 to define them as *Lactobacillus* species.

Table 1: Biochemical characters of lactobacillus isolates obtained from fermented dairy products

S.N.	Isolates	Catalase	IMViC Test	Starch Hydrolysis	Carbohydrate Fermentation			
					Lactose	Sucrose	Maltose	Mannitol
1.	LB-1	–ve	–ve	–ve	+ve	+ve	–ve	+ve
2.	LB-2	–ve	–ve	–ve	+ve	+ve	–ve	+ve
3.	LB-3	–ve	–ve	–ve	+ve	+ve	–ve	+ve
4.	LB-4	–ve	–ve	–ve	+ve	+ve	+ve	+ve
5.	LB-5	–ve	–ve	–ve	+ve	+ve	+ve	+ve
6.	LB-6	–ve	–ve	–ve	+ve	+ve	–ve	+ve
7.	LB-7	–ve	–ve	–ve	+ve	+ve	+ve	+ve
8.	LB-8	–ve	–ve	–ve	+ve	+ve	+ve	+ve
9.	LB-9	–ve	–ve	–ve	+ve	–ve	+ve	+ve
10.	LB-10	–ve	–ve	–ve	+ve	+ve	–ve	+ve
11.	LB-11	–ve	–ve	–ve	–ve	+ve	+ve	+ve
12.	LB-12	–ve	–ve	–ve	+ve	+ve	+ve	+ve
13.	LB-13	–ve	–ve	–ve	+ve	+ve	–ve	+ve
14.	LB-14	–ve	–ve	–ve	+ve	+ve	–ve	+ve

According to the results of biochemical tests as described in table 1 the probability of LAB would have been *L. acidophilus, L. fermentum, L. plantarum, L.casei, L. ruteri* or *L. rhamnosus* that may coincide the investigations of other researchers [9]. Almost all the bacterial isolates were negative of the catalase, IMViC and starch hydrolysis tests which in general the common character of lactobacillus species with some exceptions where all the isolates have the ability to utilize the lactose, sucrose, maltose and mannitol like carbohydrates. The isolates designated as LB-1, LB-2, LB-3, LB-6, LB-10, LB-13 and LB-14 were negative of maltose utilization out of the 14 bacterial isolates. The growth on selective media, colony morphology, and microscopy and biochemical characters confirms that the isolated bacterial isolates were of Lactobacillus species[10].

Antimicrobial Potential

It was observed from the results that the antimicrobial potential of fermentation extracts was not similar towards all the test microbial species (Table 2). Only selected microbial species imparted antimicrobial activity through unknown substances secreted within fermentation extract. Out of the 14 indigenously isolated Lactobacillus spp. the isolates LB-2, LB-3, LB-5, LB-6, LB-7, LB-9, LB-11, LB-12, LB-13 and LB-14 were reported to have inhibitory potential towards test microbial species used in present work through their fermentation extract. The extracts of LB-2 was inhibitory towards *E. coli*, *P. aeroginosa* and *S. aureus* where LB-7 was reported to inhibit the *E. coli*, *S. aureus* and *C. albicans*but not the *P. aeroginosa.* Extracts of isolates Lb-3, LB-6, and LB-13 were reported to inhibit *P. aeroginosa*, and *S. aureus* respectively while LB-14 extract was inhibitive towards *E. coli* only.

Table 2: Antimicrobial potential of the fermentation extract of indigenous *Lactobacillus* isolates

S.N	Isolates	Antimicrobial potential against			
		E. coli **(MTCC-1687)**	***P. aeroginosa*** **(MTCC-*1934)**	***S. aureus*** **(MTCC-737)**	***C. albicans*** **(MTCC-227)**
1.	***LB-1***	–ve	–ve	–ve	–ve
2.	***LB-2***	+ve	+ve	+ve	–ve
3.	***LB-3***	–ve	+ve	–ve	–ve
4.	***LB-4***	–ve	–ve	–ve	–ve
5.	***LB-5***	+ve	–ve	–ve	+ve
6.	***LB-6***	–ve	–ve	+ve	–ve
7.	***LB-7***	+ve	–ve	+ve	+ve
8.	***LB-8***	–ve	–ve	–ve	–ve
9.	***LB-9***	–ve	–ve	–ve	+ve
10.	***LB-10***	–ve	–ve	–ve	–ve
11.	***LB-11***	+ve	–ve	–ve	+ve
12.	***LB-12***	+ve	–ve	–ve	–ve
13.	***LB-13***	–ve	–ve	+ve	–ve
14.	***LB-14***	+ve	–ve	–ve	–ve

Image 1: Antimicrobial susceptibility assay of fermentation extracts prepared from activities on indigenous Lactobacillus isolates against *E.coli*.

Image 2: Antimicrobial susceptibility assay of fermentation extracts prepared from activities on indigenous Lactobacillus isolates against *P. aerogenosa*.

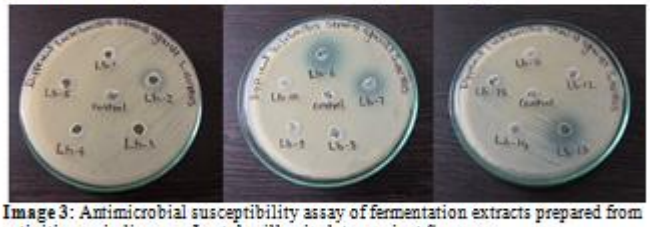

Image 3: Antimicrobial susceptibility assay of fermentation extracts prepared from activities on indigenous Lactobacillus isolates against *S. aureus*.

Image 4: Antimicrobial susceptibility assay of fermentation extracts prepared from activities on indigenous Lactobacillus isolates against *C. albicans*.

Figure 1: Antimicrobial activity of fermentation extracts of indigenously isolated *Lactobacillus* spp. against MTCC cultures used in present study.

From the results as depicted in table 2 for antimicrobial activity of fermentation extracts of indigenously isolated Lactobacillus species indicates that all some inhibitory substance must be secreted by these LAB species which are differently inhibitive towards the varied pathogenic test microbial species but any how promises to develop antimicrobial substances of probiotic origin that could be used in variety of ways in biopharmaceuticals, foods, cosmaceuticals and other uses. Finding out the antagonistic substances against the pathogenic microbial species would have been the great efforts of scientists if it comes from natural origin and natural substances instead to synthetic and harmful drugs[11-13]. Probiotic bacteria beneficially affect human health by improving the gut micro biota balance and the defenses against pathogens. Additional health benefits attributed to probiotics are the stimulation of the immune system, blood cholesterol reduction, vitamin synthesis, anti-carcinogenesis and anti-bacterial activities [14]. Probiotic

bacteria produce lactic acid and organic acids, reduce the pH environment, and try to prevent the growth of many bacteria. These bacteria produce antimicrobial compounds such as bacteriocin which can be used as natural preservatives[15].

CONCLUSION

From the results of present investigation it could be concluded that the dairy products that are being in the state of fermentation bears the load of naturally beneficial Lactic acid bacteria that could be used production of several natural substances of bacterial origin by the standardized process of fermentation. The indigenously isolated microbial of probiotic nature since they belongs to the genus Lactobacillus have the ability to produce antagonistic substances during the process of fermentation and their extracts have the ability to inhibit certain pathogenic microbial species that are responsible for food contamination in domestic or industrial system. The antagonistic substances could be explored extensively for identification, purification and use in food preservation at both domestic as well as industrial levels.

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