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Probiotic potentials of lactic acid bacteria isolated from milk of domestic animals

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ABSTRACT

Probiotics are the health promoting viable microorganisms that exhibit a beneficial effect on the health of the host on ingestion viable microorganisms. For the isolation of *Lactobacillus* strains with probiotic potentials, a total of 65 milk samples (25 from buffalo and 40 from cow milk) were analyzed and 44 isolates were identified as Lactic Acid Bacteria (LAB) on the basis of morphological, physiological and biochemical characteristics. These LABs were tested for antimicrobial potentials against *E.coli* (MTCC 443), bile tolerance and acid tolerance and 16 isolates passed these tests satisfactorily. These isolates included *Lactobacillus plantarum (*45%), *L.acidophilus* (20%), *L.bulgaricus* (12%), *L.lactis* (12%) and *L.rhamnosus* (12%). The data showed that these LAB isolates could be used as potential probiotics from the milk of domestic animals for food fortification.

KEYWORDS

Probiotics;
Lactic acid bacteria*;*
Acid tolerance;
Bile tolerance;
Antibacterial activity.

INTRODUCTION

The food is not only for energy or palatability but also for sustaining the health of individual human being. In this context, the recent trend is to develop food with probiotics (the health promoting viable microorganisms that exhibit a beneficial effect on the health of the host on ingestion) is promoted[11,13]. Milk and milk products are usually associated with probiotic bacteria, which provide supplements in maintaining beneficial intestinal balance[9]. Probiotics control intestinal pathogens by production of antibacterial compounds, including lactic and acetic acid and antibiotic-like substances, competition for nutrients and adhesion sites, increased and decreased enzyme activity, increased antibody levels and increased macrophage activity. Lactic acid bacteria (LAB) have been used successfully, with few adverse effects, to prevent antibiotic associated diarrhea, to treat acute infantile diarrhea and recurrent *Clostridium difficile* disease and to treat various diarrheal illnesses. The antagonistic property is attributed to the lowered pH, the undissociated acids and production of other primary and secondary antimicrobial metabolites produced by LAB. The metabolites produced by the fermentation process, except the volatile ones, are kept in the foods and result in growth inhibition of food spoilage or poisoning bacteria and detoxification of noxious compounds of plant origin. The primary antimicrobial effect exerted by LAB is the production of lactic acid and reduction of pH. In addition, LAB produce various antimicrobial compounds, which can be classified as low-molecular-mass (LMM) com-

pounds such as hydrogen peroxide (H_2O_2), carbon dioxide (CO_2), diacetyl (2,3-butanedione), uncharacterized compounds, and high-molecular-mass (HMM) compounds like bacteriocin. All of these can antagonize the growth of some spoilage and pathogenic bacteria in foods[3].

The most widely used probiotic bacteria are *Lactobacilli* and *Bifidobacteri*a, which can survive in the intestine. The effectiveness of selected *Lactobacillus* strains used, as probiotics are to prevent and treat infectious bacterial and viral diarrhea[5]. Probiotic strains which, when consumed in appropriate amounts in food, confer a health benefit on the host. Probiotics are resistance to gastric acidity and bile salts, as based on the both survival and growth studies[4]. The probiotic bacteria must be a normal inhabitant of the gut, tolerant to acid and bile salt and exhibit antimicrobial activity against pathogenic microorganisms. The purpose of this work was to isolate strains of *Lactobacillus* spp. from milk of domestic animals in Amravati city and to evaluate the potential of *Lactobacillus* spp. as a probiotic culture by characterizing the acid and bile salt tolerance as well as antibacterial activity exhibited by these strains *in vitro*.

MATERIALS AND METHODS

Sources of isolates

Probiotic organisms *Lactobacilli* were isolated from milk of domestic animals such as 25 buffalo's milk and 40 cows milk's samples, which were randomly collected in sterilized glass bottles from different parts of Amravati city (Maharashtra State, India).

Isolation and identification of lactic acid bacteria

Milk samples were serially diluted upto 10^{-5}-10^{-6} with sterile distilled water. By appropriate dilutions, 0.1 mL diluted milk sample was plated on to sterile de-Mann Ragosa and Sharpe (MRS) agar. The plates were allowed to set and overlaid with approximately 5 mL of MRS agar to each plate to maintained microaerophilic condition and incubated at 37^{0}C for 48h. After incubation, well-isolated typical colonies were picked up randomly and transferred to MRS broth and incubated at 37^{0}C for 48h. The isolates were identified using standard morphological, cultural, physiological test and sugar fermentation pattern[8].

Detection of antibacterial activities

The antibacterial activity of isolated *Lactobacillus* species were determined by modifying the disc diffusion method. Sterile blotting paper 10 mm discs (Whatman No. 1) were dipped into the culture broth of isolated *Lactobacillus* spp. incubated for 48h and placed on solidified Nutrient agar seeded with 3h culture of test microorganisms *Escherichia coli* (MTCC 443). The plates were kept at 4^{0}C for 1h for diffusion and incubated at 37^{0}C for 24h zones of inhibition were measured.

Acid tolerance

To study acid tolerance of isolates, MRS broth was adjusted to various pHs such as 2, 3, 4 and 5 by using 0.1 N HCl and sterilized. Isolated *Lactobacillus* spp were inoculated into various MRS media adjusted to various pH and incubated at 37^{0}C for 48h. Then 0.1 mL inoculum was transferred to MRS agar by pour plate method and incubated at 37^{0}C for 48h. If growths were observed on MRS agar plate, the LAS were designated as acid tolerant isolates.

Bile salt tolerance

To study Bile salt tolerance, MRS broth was supplemented with 0.5%, 1.0%, 1.5% and 2.0% bile salt. The MRS broth with various concentrations of bile salt were inoculated with the isolated *Lactobacillus* spp. and incubated at 37^{0}C for 48h. After incubation 0.1 mL inoculum was transferred to MRS agar by pour plate method and incubated at 37^{0}C for 48h. If colonies were observed on MRS agar plate, they were designated as bile salt tolerance isolates.

RESULTS AND DISCUSSIONS:

In present study, for isolation of *Lactobacillus* spp. with probiotic potentials, a total of 65 milk samples (25 buffalo milk and 40 cow milk) were analysed and a total of 44 isolates were identified as *Lactobacillus* spp. by using standard morphological, cultural, physiological test and sugar fermentation pattern[8].

These 44 isolates were identified as *Lactobacillus casei* (12 isolates, 27%), *L.lactis* (10 isolates, 23%), *L.plantarum* (8 isolates, 18%), 4 (9%) each of

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TABLE 1: Antagonistic activities against *E.coli*, acid tolerance, bile salt tolerance and probiotic potentials of LAB isolates from milk of buffalos and cows

Lactobacilli Isolates	*Code*	*Zone in inhibition against E.coli*	Acid tolerance (pH)				Bile salt tolerance (%)				Probiotics
			5	4	3	2	0.5	1.0	1.5	2.0	
L.acidophilus	Sab.20	18 mm	+	+	+	+	+	+	+	+	+
L.acidophilus	Sab.26	18 mm	+	+	+	+	+	+	+	+	+
L.acidophilus	Sab.8	15 mm	+	+	+	+	+	+	+	+	+
L.bulgaricus	Sab.5	16mm	+	+	+	+	+	+	+	+	+
L.bulgaricus	Sab.1	*15 mm*	+	+	+	+	+	+	+	+	+
L.lactis	Sab.11	16 mm	+	+	+	+	+	+	+	+	+
L.lactis	Sab.7	15 mm	+	+	+	+	+	+	+	+	+
L.plantarum	Sab.23	18 mm	+	+	+	+	+	+	+	+	+
L.plantarum	Sab.27	17 mm	+	+	+	+	+	+	+	+	+
L.plantarum	Sab.6	16 mm	+	+	+	+	+	+	+	+	+
L.plantarum	Sab.9	16 mm	+	+	+	+	+	+	+	+	+
L.plantarum	Sab.10	16 mm	+	+	+	+	+	+	+	+	+
L.plantarum	Sab.15	*16 mm*	+	+	+	+	+	+	+	+	+
L.plantarum	Sab.4	14 mm	+	+	+	+	+	+	+	+	+
L.rhamnosus	Sab.25	19 mm	+	+	+	+	+	+	+	+	+
L.rhamnosus	Sab.19	18 mm	+	+	+	+	+	+	+	+	+
L.bulgaricus	Sab.18	17 mm	+	+	+	-	+	+	+	+	-
L.bulgaricus	Sab.24	17 mm	+	+	+	-	+	+	+	-	-
L.casei	Sab.2	14 mm	+	+	+	-	+	+	+	-	-
L.casei	Sab.12	15 mm	+	+	+	-	+	+	+	+	-
L.casei	Sab.13	15 mm	+	+	+	-	+	+	+	-	-
L.casei	Sab.17	17 mm	+	+	+	-	+	+	+	-	-
L.casei	Sab.22	17 mm	+	+	+	-	+	+	+	-	-
L.lactis	Sab.3	14 mm	+	+	+	-	+	+	+	-	-
L.lactis	Sab.16	15 mm	+	+	+	-	+	+	+	-	-
L.lactis	Sab.21	18 mm	+	+	+	-	+	+	+	+	-
L.rhamnosus	Sab.14	15 mm	+	+	+	-	+	+	+	+	-

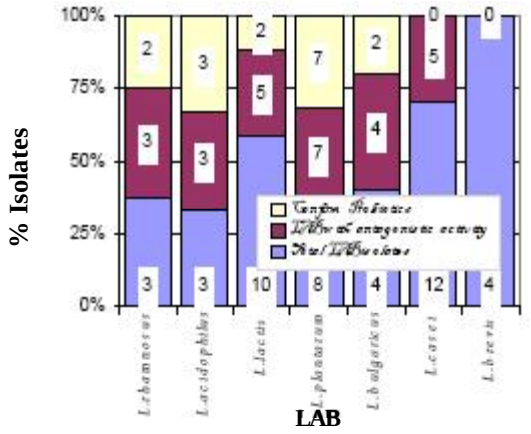

Figure 1: Lactobacilli isolated from buffalos and cow milk

L.bulgaricus and *L.brevis* and 3 (7%) each of *L.acidophilus* and *L.rhamnosus*. (Figure 1). Guessas and Kihal (2004) had isolated *L.*species from raw goat's milk such as *L.casei, L.cruvatus, L.reuteri, L.brevis, L.bulgaricus, L.paracasei, L.helveticus, L.acidophilus, L.plantarum*.

Antibacterial activity

The antibacterial activity of these isolates was determined against *E.coli* (MTCC 443). Out of these 44 isolates, a total of 27 isolates showed strong inhibition in the disc diffusion test against *E.coli*, which includes *L. rhamnosus* (3), *L. acidophilus* (3), *L.lactis* (5), *L.plantarum* (7), *L.bulgaricus* (4), and *L.casei* (5). Among the isolates, *L. plantarum* was dominant species and *L. rhamnosus* showed the maximum (Zone of inhibition of growth 19 mm in 24h) antibacterial potential against *E.coli* (TABLE 1). This may be due to the production of acetic and lactic acids that lowered the pH of the medium or competition for nutrients, or due to production of bacteriocin or antibacterial compound[10].

Acid and bile salt tolerance

It is important that the probiotic microorganisms are able to reach the GIT and remain viable in stomach at pH around 1 to 2. Bile tolerance is one of the most essential criteria for strains to be used as probiotic. Tolerance to bile salts is considered to be prerequisite for colonization and metabolic activity of bacteria in the small intestine of the host. Bile acids are inhibitorier than organic acids in gut. Therefore, when evaluating the potential LAB as effective probiotics it should passed the resistance of bile acids[13].

Twenty-seven isolates, which showed antibacterial activity, were further screened for acid and bile salt tolerance (TABLE 1). These 27 isolates of *Lactobacillus* spp. were survived at 0.5%, 1.0% and 1.5% bile salt concentrations. Among them 20 isolates of *Lactobacillus* spp. were showed highest tolerance at 2% bile salt concentration (TABLE 1). The 2% bile salt used for testing these strains represents the extreme concentration obtained in animal or human intestine during the first hour of digestion[6]. Results demonstrated that with longer incubation times, bile tolerant isolates grew, which is contrary to results of Lankaputhra and Shah[12]. Before reacting the intestinal tract, probiotic bacteria must first survive transit through the stomach where the pH can be as low as 1.5 to 2[2]. Acid tolerance of these

isolates was investigated at various pH values. These 27 isolates of *Lactobacillus* spp. were survived at 5,4 and 3 pH. The study showed that a total of 16 LABs were acid tolerance at pH 2, and bile salt tolerance at 2% (Figure 1). The isolates include *L.plantarum* (45%), *L. acidophilus* (20%), *L.bulgaricus* (12%), *L.lactis* (12%) and *L.rhamnosus* (12%). This proves that these LABs strains can be worked as probiotics by surviving in high acidic environment in the stomach and high of bile salts concentration in the intestine.

CONCLUSION

Isolated *Lactobacillus* spp. exhibited good probiotic characteristics that might be used for food or dairy fermentations and contribute health benefits to consumers. The study suggested that probiotics are helpful in the protection and improvement of intestinal flora. These isolated LABs can help to stabilize the gut microbial environment and the intestine's permeability barrier, there by promoting the immunological barrier to gut mucosa and also holds great promise for the prevention and treatment of clinical conditions associated with impaired gut mucosal barrier functions and sustained inflammatory responses. Probiotics approach is to reconstitute natural condition by means of repairing a deficiency neither by addition of foreign chemicals to the body, which may have toxic consequences or, as in the case of antibiotics induce resistance and compromise subsequent therapy. Thus use of these isolated LABs can used as potential probiotic for combating various aliment of human being.

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