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Research Article

Assessment of Lactic Acid Bacteria from Cow Milk and Goat Milk Samples for Probiotic Potentiality by *In Vitro* Methods

Amir Mahalot and Shyamapada Mandal*

Laboratory of Microbiology and Experimental Medicine, Department of Zoology, University of Gour Banga, Malda, West Bengal, India

***Corresponding Author:** Shyamapada Mandal, Professor, Laboratory of Microbiology and Experimental Medicine, Department of Zoology, University of Gour Banga, India. **E-mail:** samtropmed@gmail.com

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Abstract

As reported, the milk and milk based fermented food are the well-known niches for the growth of lactic acid bacteria (LAB). This communication aims to isolate LAB from locally available raw milk samples from cow and goat, and to assess the probiotic attributes of isolated LAB. The three freshly collected milk samples (cow milk: 1 and goat milk: 2) were subjected to microbiological analysis for the isolation of LAB. The milk isolates of LAB were studied phenotypically, following cultural, morphological, physiological and biochemical characterization, for their identity. The probiotic attributes (tolerance to high range of temperature and sodium chloride concentrations and low-pH, and tolerance to bile salts) and safety profiling (haemolysis and gelatin hydrolysis patterns and susceptibility to antibiotics) was done following standard protocols. The LAB (n = 3) isolated, one from each of the milk samples, were homo-fermentative lactobacilli (n = 2): *Lactobacillus* sp. G1 and *Lactobacillus* sp. C1, and *Lactococcus* sp. G2; all of which well tolerated low-pH (3.0 - 2.5), bile salts (0.2 - 0.3), temperature (4^0C - 42^0C) and sodium chloride (2% - 6%). The LAB isolates were sensitive to most of the antibiotics tested, except methicillin (for all isolates), trimethoprim (for *Lactobacillus* sp. G1 and *Lactococcus* sp. G2) and vancomycin (for *Lactobacillus* sp. G1 and *Lactobacillus* sp. C1). The locally available cow milk and goat milk are excellent source of LAB, and, based upon the probiotic attributes and safety profiles, the isolated LAB might be utilized by the consumers, at least in our part of the globe, for health benefits.

Keywords: Lactic Acid Bacteria (LAB); Cow Milk; Goat Milk; Probiotics

Introduction

Lactic acid bacteria (LAB) isolated from milk and milk-based as well as non-milk based fermented foods have been conferred the GRAS (generally recognized as safe) status and have widely been used in food and medicine, because of their probiotic attributes. The LAB strains are associated to many health benefits of which balancing the gut microbiota remains the vital one. Among LAB, members of the genus *Lactobacillus* and *Lactococcus* constitute the major probiotics available in the globe. In our previous studies we have procured different probiotic *Lactobacillus* isolates: *Lactobacillus fermentum*, and *Lactobacillus casei, Lactobacillus animalis, L. acidophilus, L. plantarum* and *L. rhamnosus* from homemade and commercially available curd samples [1,2]. A large number of LAB, including *L. plantarum, L. rhamnosus, L. delbruckii, L. fermentum* and *L. pentosus* from cow milk and goat milk samples have been characterized for probiotic justification considering tolerance to various stressors, such as bile salt, low-pH, sodium chloride, temperature, and safety profiling through hemolytic patterns, gelatin hydrolysis capacity and antibiotic susceptibility [2-4]. Such probiotic LAB possesses the capacity to produce lactic acid, hydrogen peroxide and bacteriocins responsible for antagonizing various pathogenic bacteria, as has been reported by the scientists over the globe, time to time [2,5,6]. However, no scientific report was available, from our geographic region, about the isolation and characterization of LAB from cow- and goat-milk samples available from local niches. Therefore, the current study has been aimed to isolate LAB from locally available raw milk samples from cow and goat and to assess the probiotic attributes of isolated LAB.

Materials and Method

Cow milk and goat milk samples

A total of three fresh milk samples (cow milk: 1, and goat milk: 2) were collected from cow and goat sheds, from our locality, in sterilized screw capped collecting vials and transported to the Laboratory of Microbiology and Experimental Medicine, Department of Zoology, University of Gour Banga, for microbiological analyses.

Isolation of lactic acid bacteria from milk samples

In order to enrich the growth of lactic acid bacteria (LAB), milk samples (500 µl, each) were inoculated into MRS broth (Hi-Media, India), and incubated for 24 - 72 hours, at 37^0C. Subculture of LAB thus obtained was done following streak-dilution method on MRS agar (Hi-Media, India) plate, and after incubation for 48 - 72 hours, at 37^0C, single discrete colonies of LAB, developed on the MRS agar plate, were selected and stored in MRS broth (Hi-Media, India) as well as MRS agar (Hi-Media, India) stabs [1].

Characterization of lactic acid bacterial isolates

The LAB isolated were subjected to gram-staining, catalase, oxidase, indole and motility testing, and thereafter, characterized by performing biochemical and sugar fermentation tests, according to Bergey's manual [7], as described earlier [1].

Probiotic attributes of lactic acid bacteria

The probiotic features of the LAB were justified with their tolerance capacity to physiological stressors: NaCl (2% to 6%), bile salts (0.2% and 0.3%; wt/vol) and acidity (low-pH: 2.0 to 4.0), following the protocol mentioned earlier, using MRS broth [1,8,9]. The turbidity in the culture, following incubation at 37^0C for 24 hours, indicated the tolerance capacity of LAB, and the same was confirmed by the appearance of lactobacilli colonies, on sub-culturing the 24 hours grown broth culture after stressors treatment, on MRS agar plate.

Safety profiling

The safety profiling of the milk isolates of LAB were authenticated by haemolytic activity [2], gelatin liquefaction test using nutrient gelatin medium [Hi-Media, India], and antibiotic susceptibility testing by disc diffusion method [10], as described by Halder and Mandal [2,11]. The ZDI values obtained were interpreted according to the criteria mentioned earlier [12,13]: the LAB was grouped into resistant (ZDI: ≤ 15 mm), sensitive (ZDI: ≥ 21 mm), or intermediately susceptible (ZDI: 16 - 20 mm).

Results and Discussion

Three bacterial isolates procured: G1 and G2 from goat milk samples, and C1 from cow milk, were gram-positive, non-spore forming and showed negative test results to catalase, oxidase and indole production, and thus were regarded as LAB. The LAB strains thus isolated were very small rod (rodococcus: G2) to small rod shaped (G1 and C1) and were non-motile belonged to the genus *Lactobacillus*. Further, all the isolates were citrate and VP test negative and did not produce H_2S; the C1 was positive to MR test, while G2 produced urease, reduced nitrate and had MR test positivity. The sugar fermentation activity of the *Lactobacillus* isolates is represented in Table 1; the all LAB isolates: *Lactobacillus* sp. G1 and *Lactococcus* sp. G2 from goat-milk samples, and *Lactobacillus* sp. C1, from cow-milk sample, were homo-fermentative. The earlier study demonstrated about the identification of LAB from milk samples of domestic animals (cow, buffaloes, goat and sheep), following gram-staining, cultural characteristics, physiological properties and biochemical tests [14,15]. Forhad., *et al.* [16] isolated LAB (*L. fermentum, L. casei, L. acidophilus*) and identified the isolates, with biochemical analysis, which had reliable probiotic features.

The LAB to be measured as probiotics, the strains/isolates are required to possess the capacity to survive at pH 3.0 as well as in presence of bile salt, at 0.1% [3,17]. Narwade., *et al.* [18] characterized *Lactobacillus* isolates from fresh cow milk samples, however, did not report their probiotic features. Mithun., *et al.* [19] utilized cow and buffalo milk samples to isolate *L. fermentum, L. acidophilus, L. viridescens, L. brevis* and *L. gasseri,* with a concord for probiotic characterization. A number of LAB have been procured from milk samples from cow and goat and were justified as probiotics, of which the goat milk isolate, *L. plantarum* G8 was found as the most competent one [20]. The potential probiotic LAB isolates: *L. rhamnosus, L. plantarum* and *L. plantarum* from goat milk samples, grew well in acidic condition (pH 2.0, 2.5, and 3.2), and survived in presence of bile salt (0.3%), as per the report of Setyawardani., *et al.* [21]. In the current study, the LAB isolates (*Lactobacillus* sp. G1, *Lactobacillus* sp. C1 and *Lactococcus* sp. G2), tolerated high range of temperature variation (4^0C to 42^0C), high bile salt (0.2% to 0.3%) and NaCl (2% to 6%) concentrations, and low-pH (3.0 to 2.5) condition; the isolates did not survive at pH ≤ 2.0.

The lactobacilli isolates, in the current investigation, were tested for their safety properties: haemolytic as well as gelatinase activity and susceptibility to antibiotics. The safety profiling has been suggested as one of the important attributes, as per the FAO/ WHO guidelines [22], on assessing the probiotics feature of LAB. Absence of haemolytic as well as gelatinase activity, among the indigenous lactobacilli isolates, is indicative of their non-virulence nature [23]. Herein, the cow-milk and goat-milk isolates of LAB (*Lactobacillus* sp. G1, *Lactobacillus* sp. C1 and *Lactococcus* sp. G2) showed γ-haemolysis (non-haemolytic) and negative results to the gelatinase activity test. The antibiotic susceptibility test results for the isolated lactobacilli are represented in Table 2. All the test lactobacilli had resistance to Mc, and *Lactobacillus* sp. G1 and *Lactobacillus* sp. C1 had Vm resistance, while Tm resistance was detected in *Lactobacillus* sp. G1 and *Lactococcus* sp. G2. As has been reported by Sieladie., *et al.* [3], the acid and bile tolerant lactobacilli isolates from fresh cow milk had sensitivity to all, except Cm, of the antibiotics tested.

Sugars	LAB strains		
	G1	G2	C1
Dextrose	+	+	+
Sucrose	+	+	+g
Xylose	+	-	w
Rhamnose	-	-	-
Raffinose	+g	-	W
Cellobiose	-	-	-
Mannitol	-	W	-
Lactose	+	+	+
Melezitose	-	-	-
Mannose	-	+	-
Fructose	+g	+	+g
Ribose	+	-	+
Salicin	-	-	-

Table 1: Sugar fermentation test results for milk isolates of lactic acid bacteria.

+: Positive; –: Negative; W: Weakly Positive; g: Gas Production.

Strain	R (ZDI: ≤15 mm)	IS (ZDI: 16 – 20 mm)	S (ZDI: ≥ 21 mm)
Lactobacillus sp. G1	Mc: 6; Vm: 6; Tm: 6	Ac: 18; Ak: 20	AS: 26; Cp: 22; Cm: 26; Cx: 32; Tc: 26
Lactococcus sp. G2	Mc: 13; Tm: 6	Ak: 20; Vm: 18	AS: 35; Ac: 36; Cx: 47; Tc: 38; Cp: 30; Cm: 36
Lactobacillus sp. C1	Mc: 6; Vm: 6	Ak: 17;	AS: 20; Ac: 21; Tm: 30; CTX: 22; Tc: 25; Cp: 20; Cm: 22

Table 2: Antibiotic susceptibility test results for LAB isolates from milk samples.

Ak: Amikacin; Ac: Amoxyclav; AS: Ampicillin-Sulbactam; Cx: Cefotaxime; Cm: Chloramphenicol; Cp: Ciprofloxacin; Mc: Methicillin; Tm: Trimethoprim; Tc: Tetracycline; Vm: Vancomycin; IS: Intermediately Susceptible; R: Resistant; S: Sensitive; LAB: Lactic Acid Bacteria.

Conclusion

The results of the instant study suggest as well as authenticate the cow milk and goat milk, from locally available niches, as the potential sources of lactic acid bacteria (*Lactobacillus* and *Lactococcus*) to be utilized as potential probiotics.

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