

**THESIS ABSTRACT**



**CHARACTERIZATION OF LACTIC ACID BACTERIA ISOLATED FROM GOAT AND CAMEL MILK AND THEIR EFFECT ON BACTERIAL PATHOGENS**

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**ABSTRACT**

Milk due to its high nutritive content, can support a rich micro-biota with varied properties; some facilitate dairy fermentations, some cause spoilage, some promote health, others cause diseases. Lactic Acid Bacteria (LAB), generally regarded as safe (GRAS) are probiotic in nature. GRAS, due to their pro-health benefits is of particular importance. They prevent food spoilage and are known to alleviate lactose intolerance, peptic ulcers, and diarrhea, thus, boosting the intestinal functions. They also stimulate the immune system, prevent colon cancer, have anti-allergic and antifungal effects, and can influence brain functions, viz., depression and anxiety as well.

Goat and camel are animals of arid regions and survive adverse climatic conditions with extreme temperatures and water scarcity. The goat and camel milk are a rich source of many vitamins, minerals and are abundant in proteins with remarkable intrinsic therapeutic properties. Milk from these animals can be consumed even by lactose intolerant people. Camel milk does not contain  $\beta$ -lacto globulin and contains non allergen A2 subtype beta casein compared to A1 beta casein in cow's milk. The present study was planned with a clear hypothesis that the probiotic lactic acid bacteria isolated from camel and goat milk will have stable growth and enhanced antimicrobial property as these animals survive extreme climatic conditions and also their milk has inherent unique properties. The entire study has been organized into the following three chapters.

**Assessment of Milk for food borne pathogenic bacteria and isolation of Probiotic Lactic Acid Bacteria:** A total of 168 milk samples of goat and camel milk were examined comprising 106 from goats and 62 from camels collected during the three seasons over two consecutive years from four different breeds of goats and camels. Milk samples were enriched for lactic acid bacteria (LAB) in selective media MRS (de Man Rogosa Sharpe).

Occurrence of LAB was confirmed from 60.4% of goat milk samples and 29% of the camel milk samples; while samples collected in summer season showed increased bacterial load. Statistical analysis shows a significant difference ( $p \leq 0.05$ ) in the occurrence of LAB in milk samples collected in different seasons but not in the samples collected from different breeds.

Isolation of other food-borne bacteria, viz., *E. coli*, *Salmonella* and *Staphylococcus* was done from these samples. These bacteria are responsible for food-borne illnesses and have been reported to be a cause of many outbreaks around the world. Statistical analysis showed a significant difference ( $p \leq 0.05$ ) in the occurrence of these three pathogenic bacteria in the samples of milk collected from different breeds in different seasons. Thus, it is obvious that environmental conditions directly affect the milk composition and the indigenous micro-biota in milk. The 360 confirmed LAB isolates were subjected to screening for their antimicrobial activity.

The indicator strains used were the five major food borne pathogens, viz., *Escherichia coli*, *Salmonella typhimurium*, *Staphylococcus aureus*, *Listeria monocytogenes* and *Cronobacter sakazakii*. Only 18 isolates showed broad spectrum antimicrobial property and checked the growth of all the five pathogenic bacteria (gram positive and gram negative). 11 LAB isolates were from goat milk and 7 from camel milk. Co-occurrence of the food borne pathogenic bacteria was not found in the samples from which these 18 probiotic LAB were isolated. These isolates were further characterized biochemically and molecularly.

**Characterization of Lactic Acid Bacteria:** The antibiograms (Antibiotic susceptibility patterns) of LAB did not show any specific distinguishing feature amongst the isolates. All the isolates were found susceptible to Penicillin, Methicillin, Oxacillin, Erythromycin, Ampicillin, Gentamicin, Chloramphenicol, and Clindamycin. LAB isolates were resistant to Vancomycin, Teicoplanin, Ceftazidime, Cefoxitin, Ciprofloxacin, Fosfomycin and Nitrofuratoin. The susceptibility for different isolates as recorded showed variation.

The whole cell protein profiling of the selected 18 isolates was conducted on SDS PAGE. A distinct difference was observed in protein patterns of isolates from goat and camel milk. Further, for identification, the LAB isolates were grouped into five groups based on their physiological and biochemical behavior. Cell morphology, growth at different temperatures, media pH and media salt concentrations were studied along with mode of glucose fermentation and sugar fermentation profiles to divide the isolates into homo-fermentative *Enterococcus*, *Lactococcus*, *Pediococcus*, *Lactobacillus* and hetero-fermentative *Leuconostoc*.

DNA was extracted and RAPD (Random Amplified Polymorphic DNA) was performed using primer M13 (5' – GAG GGT GGC GGT TCT – 3'). 16S rRNA gene was amplified to identify the isolates through sequencing. The isolates were confirmed as *Pediococcus acidilactici*. The sequences were submitted to NCBI GenBank and the accession numbers obtained were KY411915, KY411916, KY41827 and KY508242.

**Optimization of Antimicrobial Activity of Lactic Acid Bacteria:** Antimicrobial activity was determined by the agar-well-diffusion method for cell cultures, cell free supernatant and bacteriocin containing supernatant under varying media composition and culture conditions.

The optimum conditions for antimicrobial activity and bacteriocin production were incubation at 37°C for 18 hours, in a media with pH 6.5, containing dextrose or mannose as the carbohydrate sources. Increasing Tween-80 conc. and NaCl conc. also enhanced the inhibition zones showing an increased antimicrobial activity in-vitro.

The deliverables of the study are that the partially purified bacteriocin may prove to be a good bio-preservative and a good substitute for antibiotics. The culture itself may be implemented as a probiotic starter culture and even introduced in marketed dairy products and other fermented products. The antimicrobial potential and stability of these isolates may find their use in industries and medical care.

**CONCLUSION:** The findings of the present study are important to various industrial and medical applications. The present study is a humble step towards a systematic research approach to utilize LAB from camel and goat milk. Further, molecular and analytical research is required to apply this concept of immense therapeutic and commercial value.

**KEY WORDS:** Antibiogram, Bacterial pathogens, Camel milk, Lactic acid Bacteria, Goat milk

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**UNDERTAKING**



**Dr (Miss) Mahima Verma**

It is certified that the thesis entitled '**CHARACTERIZATION OF LACTIC ACID BACTERIA ISOLATED FROM GOAT AND CAMEL MILK AND THEIR EFFECT ON BACTERIAL PATHOGENS**' is an original research work carried out by me in the Dept. of Zoology, Faculty of Science, Dayalbagh Educational Institute (Deemed University), Agra, under the supervision of Professor Alka Prakash (Guide) and Professor Sant Prakash (Co-Guide) ), and has been admitted for the award of the Degree of Doctor of Philosophy in Zoology (December 2017).



**SCHOLAR**