Introduction

Milk being a source of many nutrients forms an important part of diet all over the world. Attributes of milk differs from place to place and from animal to animal. Although milk from cow and buffalo are most commonly consumed, but milk from goat, camel, ewes, yak and others also form a considerable share in total milk being consumed over the world. In the present study we are taking into account the properties of goat and camel milk as these animals survive in adverse climatic conditions and form a major part of food in arid regions. Comparative studies on milk from these two animals have not yet been reported. Although work has been done individually on them but still there is lot to explore. Besides this, study may produce interesting results with respect to their therapeutic value.

Goat's milk is a very good source of calcium and the amino acid tryptophan. It is also a good source of protein, phosphorus, riboflavin (vitamin B2) and potassium. Perhaps the greatest benefit of goat's milk, however, is that some people who cannot tolerate cow's milk are able to drink goat's milk without any problems.

Camel milk is a staple food of desert nomad tribes and is considered a whole food as it's rich in vitamins, minerals, proteins and immunoglobulins. Normal camel on good feed can produce up to 2000 liters of milk per lactation period which is much higher than cow. In spite of this, information about camels as milk animal is very limited. Camel milk is consumed fresh as the rate of fermentation is very high in it and this could be the possible reason for its limited use in spite of it being a healthy diet.

Diverse studies have been carried out on different aspects of correlation of pathogenic bacteria and various milk products. The microbiological quality of milk and milk products is influenced by the initial flora of raw milk (Ritcher & Vadamuthu, 2001). Searching for desirable microbial strains for the food industry, isolation of microorganisms from traditional fermented products and characterization of physiological properties are a constant effort of scientific communities around the world (Steinkraus et al., 2002). Therefore, analysis of lactic acid fermentation processes is a necessary step, especially in products where changes in microbiological composition have been detected (Parente & Ricciardi, 1999).

Since ages, lactic acid bacteria have been widely put-to-use in domestic fermentation of food such as yogurt, cheese, pickles, etc. In 1873, ten years after Louis Pasteur studied lactic acid fermentation (between 1857 and 1863) the first pure culture of a lactic acid bacterium (LAB) ("Bacterium lactis") was
obtained by J. Lister. The use of LAB in food fermentations, their probiotic potential, and in some
instances their severe pathogenicity have been the key reasons driving research in this group of bacteria.

The lactic acid bacteria (LAB) in general are referred to related group of far and wide present
Gram-positive bacteria which produce lactic acid as the major metabolic end-product of carbohydrate
fermentation. A typical lactic acid bacterium grown under standard conditions (non-limiting glucose
concentration, growth factors and oxygen limitation) is gram-positive, non-sporing, catalase negative in
the absence of porphorinoids, aerotolerant, acid tolerant, organotrophic, and a strictly fermentative rod or
coccus, producing lactic acid as a major end product. It lacks cytochromes and is unable to synthesize
porphyrins.

LABs are part of the healthy microbiota of the human gut. Apart from dental caries, lactobacilli are
generally considered apathogenic. Lb. plantarum could be associated with endocarditis, septicemia and
abscesses.

Today, LAB are a focus of intensive international research for their essential role in most fermented
food, for their ability to produce various antimicrobial compounds promoting probiotic properties
(Temmerman et al., 2002) including antitumoral activity (De Vuyst & Degeest, 1999; Hilde et al., 2003),
reduction of serum cholesterol (Jackson et al., 2002), alleviation of lactose intolerance (De Vrese et al.,
2001), stimulation of the immune system (Isolauri et al., 2001), stabilization of gut microflora (Gibson et
al., 1997). LAB strains that produce exopolysaccharide (ESP) are employed in the manufacture of
fermented milk to improve its texture and viscosity (Curk et al., 1996; Ruas-Madiedo et al., 2002). Some
LAB strains are known to produce mannitol which is claimed to have several health promoting effects
(Wood & Holzapfel, 1995; Wisselink et al., 2002).

There are more than 250 known diseases that are transmitted through food. Most of these diseases
are infections caused by a variety of bacteria, viruses, and parasites that can be foodborne. Acidification
of food by LAB inhibits the growth of spoilage agents, thus helping to increase the shelf-life of food.
The anti-microbial activity of lactic acid bacteria has been attributed to the production of metabolites
such as organic acids (lactic and acetic acid), hydrogen peroxide, ethanol, diacetyl, acetaldehyde,
acetoine, carbon dioxide, reuterin, reutericyclin and bacteriocins.

Bacteriocins were first discovered by A. Gratia in 1925. He was involved in the process of
searching for ways to kill bacteria, which also resulted in the development of antibiotics and the
discovery of bacteriophage, all within a span of a few years. He called his first discovery a colicine
because it killed Escherichia coli. Bacteriocins are proteinaceous toxins produced by bacteria to inhibit
the growth of similar or closely related bacterial strain(s). Bacteriocins are of interest in medicine because they are made by non-pathogenic bacteria that normally colonize the human body. Loss of these harmless bacteria following antibiotic use may allow opportunistic pathogenic bacteria to invade the human body.

The potential of using bacteriocins of lactic acid bacteria, primarily used as bio preservatives, represents a perspective, alternative antimicrobial strategy for continuously increasing problem with antibiotic resistance (Jagoda et al., 2010). These bacteriocins are considered more natural than the antibiotics in use and they are produced from GRAS organisms. Another strategy in resolving this problem is applying probiotics for different gastrointestinal and urogenital infection therapies. Bacteriocins have also been suggested as a cancer treatment.

Microbiology of various fermented camel milk products including shmen (Mourad & Nour-Eddine, 2006), shubat (Rahman et al., 2009), Airag and Tarag (Watanabe et al., 2008), cheese (Ahmed & Kanwal, 2004; Nanda, 2011), garris (Sulieman et al., 2006) has been studied. Some scientists have worked on one humped camel milk (Khedid et al., 2009), Moroccan camel’s milk (Benkerroum et al., 2003), Algerian dromedary (Hassaíne et al., 2007).

Studies on the effect of probiotics and bacteriocins in goat and camel with respect to the pathogenic bacteria are limited. No such work has been reported in Agra city. This work is thus proposed to fill the lacunae in this line of research.
Review of literature

In 1997 Cogan et al., isolated and identified LAB from about 35 artisanal dairy products of cow, goat and ewe’s milk. These workers even monitored Bacteriocin activity of few of those strains which was measured by the overlay (O) and agar diffusion (AD) techniques, most of which were found to be narrow spectrum. In 1998 Osmanağaoğlu et al., isolated bacteriocin from Pediococcus acidilactici F isolated from fermented sausage.

Benkerroum et al., 2000 characterized LAB and its bacteriocin isolated from jben (fresh cheese made from cow or goat’s raw milk). Attia et al., in 2001 investigated the capacity of camel skim milk to form acid curd and analyzed coagulum structure under SEM. Ozkaya et al., 2001 characterized phenotypically lactic acid bacteria isolates from Beyaz cheese made from raw ewes’ milk and examined their whole cell proteins by SDS-PAGE and even studied their technological properties.

Tserovska et al., in 2002 and Guessas &Kihal in 2004 isolated and identified LAB from goat’s milk and its products by conventional methods. In 2003 Benkerroum et al., assessed moroccan camel’s milk for its microbiological quality and in 2004 Ahmed and Kanwal prepared camel cheese starter culture from isolated LAB strains. Somkuti & Steinberg, 2003 transformed LAB for pediocin production and further in 2010 they studied pediocin production in milk by using variously mixed starter cultures.

Mourad and Eddine in 2006 studied biochemical & microbiological properties of shmen (butter from camel milk) by traditional methods and Sulieman et al., 2006 studied chemical and microbiological aspects of garris (Sudanese fermented camel milk product) and characterized the isolates phenotypically by using API 50 CHL kits. Whereas, Cho & Ki do in 2006 used 16S r-RNA sequencing to identify LAB isolated from jeotgal (sea food). Cueto et al., 2007 isolated and identified lab from fermented cow milk product suero costeño by using selective media and found the increase in number of Enterobacteriaceae during fermentation.

In 2007 Soomro &Masud examined whole cell protein profiles by SDS PAGE and Plasmid Profile of Lactic Acid Bacteria Isolated from Dahi (Fermented Milk Product) and isolated large sized plasmid ranging from 20 to 25 kb. Cheriguene et al., and Hassaine et al., in 2007 studied microbiological and technological properties of LAB from Algerian goat’s milk and camel milk respectively. Watanabe et al., in 2008 studied the diversity of lactic acid bacteria and yeasts in Airag and Tarag (traditional fermented milk products formed by cow, goat and camel milk) by 16S rRNA sequencing. Abdelbasset & Djamila in 2008 characterized bacteriocin isolated from a fermented milk product Raib.
Yateem et al., 2008 isolated LAB from unpasteurized camel milk cultured them and tested for antagonistic activity. Konuspayeva et al., 2008 measured fatty acid composition and cholesterol content of camel milk samples. Lodi et al., 2008 metabolically characterized LAB from camel milk by studying their acidifying, caseinolytic and reducing activities. Mezaini et al., 2009 characterized bacteriocin from Algerian dairy product. Rai et al., in 2009 determined fermentation efficiency and antibacterial property of LAB from tannery fleshing and extracted and characterized its bacteriocin.

Khedid et al., 2009 isolated LAB from one-humped camel milk by biochemical conventional methods whereas, Rahman et al., in 2009 characterized LAB in naturally fermented camel milk shubat (fermented product of unheated two humped camel milk) by sequencing of 16S rDNA. Mallesha et al., 2010 isolated and identified LAB from milk and some other fermented products and studied their antibacterial activity against some pathogens by well diffusion assay method.

Osman 2010 studied biochemical changes occurring due to fermentation in camel milk. Suskovic et al., 2010 proposed the use of bacteriocins as potential human and animal therapeutics because they are more natural than currently used antibiotics and are produced by GRAS lactic acid bacteria. Takeda et al., 2011 isolated LAB from various goat, cow and camel milk products and identified them on basis of 16S ribosomal DNA sequencing. Nanda et al., in 2011 did 16S rDNA sequencing, phylogenetic analysis and Technological characterization of LAB isolated from camel cheese samples in India.

In 2011 Patra et al., isolated and characterized LAB from Dahi, Chanos & Williams, 2011 from raw ewe’s milk, Qiuhua et al., 2011 from goat milk cake, Setyawardani et al., 2011 from goat milk and Agarwal & Dharmesh from cheese.
Objectives

- Sampling of goat and camel milk.
- Isolation and characterization of Lactic acid bacteria from the selected samples.
  - Morphological characterization.
  - Biochemical characterization.
  - Molecular characterization.
- Screening of the lactic acid bacteria isolates that inhibit food borne pathogenic bacteria like *Listeria, Cronobacter, Staphylococcus*.
- Extraction and characterization of bacteriocins from the screened isolates.
- *In-vivo* study of the effect of the isolated bacteriocins.
Work Plan and Methodology

### Sampling, isolation and characterization of lactic acid bacteria
- Samples will be collected aseptically, maintained and analyzed in the lab.
- Selective media will be used to obtain pure cultures of the lactic acid bacteria (Tserovska et al., 2002; Guessas et al., 2004).
- The lactic acid bacteria will be identified and confirmed morphologically (Ozkaya et al., 2001) biochemically and on molecular basis (Cheriguene et al., in 2007; Watanabe et al., in 2008; Qiuhua et al., 2011).

### Screening of lactic acid bacteria
- Standard cultures of pathogenic bacteria will be revived and maintained on selective media.
- The lactic acid bacteria isolates will be screened for their antimicrobial activity against the revived pathogens by Agar-well diffusion method or Disc diffusion method (Mallesha et al., 2010).

### Bacteriocin – extraction and characterization
- Extraction of bacteriocins from screened lactic acid bacteria isolates (Mezaini et al., 2009; Somkuti et al., 2010).
- Characterization at different temperatures, pH and effect of enzymes will be studied (Abdelbasset & Djamila, 2008; Mezaini et al., 2009).
- The bacteriocins will be molecularly characterized (Mezaini et al., 2009).

### In-vivo studies
- Inhibitory activity of the extracted bacteriocins on the pathogenic bacteria will be observed by measuring the inhibitory zones (Rai et al., 2009; Mezaini et al., 2009).
- Effect of extracted bacteriocins on infected animal model.
SCHEMATIC REPRESENTATION OF WORK PLAN

Sampling of goat and camel milk in aseptic conditions

Isolation and Identification of Lactic acid bacteria on selective media

Characterization of Isolated Samples

- Morphological Characterization
- Molecular Characterization
- Biochemical Characterization

Screening of Antimicrobial Properties of selected LAB against various food-borne Pathogens like *Listeria, Cronobacter, Staphylococcus*.

Extraction of Bacteriocins from screened samples

Purification of Extracted Bacteriocin

Bacteriocin Characterization

- Temperature
- pH
- Effect of Enzymes
- Molecular Characterization

In-vivo study

In-Vitro study