Introduction
INTRODUCTION

Microorganisms forming the first life on the earth making up a cryptobiotic crust or microbiotic layers play a twofold function as destructive agents and are advantageous globally. They engage in recreation in constructing the most awful to superior.

A human friendly bacterium plays an important role in promoting a healthy gut microfloral environment and ensuring proper digestion. Beneficial bacteria have several important functions within the colon like control of potentially pathogenic organisms, nutrient production and removal of toxins from the gut and stimulation of intestinal immune system. Beneficial bacteria that produce lactic acids are key organic acids keeping the human intestinal health in order and in right pH levels to discourage the growth of pathogenic bacteria. It aids in the digestive process by helping to digest lactose (milk sugar) and protein. It cleans the intestinal tract, purifies the colon and promotes regular bowel movements. It creates lactic acid, which balances intestinal pH. The intestines of animals contain billions of bacteria, some of which are beneficial and some of which are pathogenic. Some beneficial organisms are indigenous to human mouths that colonize the mouth preventing the pathogenic microorganisms (Vimalin Hena et al., 2009). A healthy intestine is the one, which maintains a critical balance between various groups of bacteria such as Lactobacilli, Streptococci, Clostridia, Coliforms and Bacteroides. Among such numerous intestinal microbes, which improve the intestinal microbial balance are selected as probiotics: Lactobacillus (L. acidophilus, L. johnsonii, L. gasseri, L. casei, L. rhamnosus and
L. sporogenes), Bifidobacterium (B. longum, B. breve, B. bifidium and B. infantis), and Enterococcus (E. faecalis and E. faecium). The proper term to use when referring to beneficial bacteria is probiotic.

Lactobacilli, a increasing tendency in counts than Bifidobacterium in human intestine (Gerhard Reuter, 2001) get their name (lacto) because they are able to turn milk sugar into lactic acid by which they alter the intestinal environment, making it unsuitable for unfriendly bacteria. In other words, lactobacilli do not destroy the unfriendly bacteria; they destroy their home, forcing them to leave. They portray as probiotic on the basis of its beneficial nature medicinally as it can reduce the adverse effect of chemotherapy and radiotherapy. Many probiotics are present in natural sources such as Lactobacillus in yogurt and sauerkraut. Claims are made that probiotics strengthen the immune system.

The word ‘probiotics’ was first introduced by Kollath in the year 1953. It carries the definition as "A live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance" (Fuller, 1989). Probiotics are living microorganisms, which are sometimes called friendly bacteria or just good bacteria helps in boosting the level of friendly bacteria in the gut, as opposed to antibiotics which attack and kill infectious and unwanted bacteria. These bacteria are found in intestine and research suggests that they have a range of functions, which helps to aid digestion (Tannock et al., 2000) making main the small intestine home.

Probiotics possess immune modulating effects and act as a first line of defense by preventing disruption of the mucosal barrier and gut associated lymphoid tissue dysfunction. Probiotics should not exhibit any pathogenic, toxic, allergic, mutagenic or carcinogenic reaction. They should be representative of microorganisms that are generally recognized as safe microorganisms (Fuller, 1989). It produces antibiotics and antifungal agents that prevent the growth of harmful bacteria and fungi. It provides a passive mechanism to prevent infection. It contributes to the destruction of molds, viruses and parasites. It protects us from environmental toxins such as pesticides and pollutants reduce toxic waste at the cellular level and stimulate the repair mechanism of cells. It increases the number of immune system cells thereby enhances immune response. It manufactures vitamins B6, B12, K, folic acid and
assorted amino acids. It helps to maintain healthy cholesterol and triglyceride levels. It breaks down and re-builds hormones. They aid in the break down of proteins, carbohydrates and fats in food and helps in absorption of necessary elements and nutrients such as minerals, amino acids and vitamins required for human and animals to survive.

Lactic Acid Bacteria (LAB) are a group of Gram positive bacteria, non sporing, non motile cocci or rods, which produces lactic acid as the major end product during the fermentation of carbohydrates. Most lactic acid bacteria are facultative anaerobes and they lack the enzyme catalase. LAB lack cytochromes and obtain energy by substrate level phosphorylation. They are strictly fermentative, aerotolerant or anaerobic, acidic or acidophilic and have complex nutritional requirements (Kashket, 1987).

It exerts a strong antagonistic activity against many food contaminating microorganisms as a result of the production of organic acids, hydrogen peroxide, diacetyl, inhibitory enzymes and bacteriocin (Piard and Desmazeaud, 1992) which primarily acts against other lactic acid bacteria living in the same ecological niche (Eijsink et al., 2002) or microorganisms that are closely related to the producer strain (De Vuyst and Vandamme, 1994). Certain lactobacilli synthesize antimicrobial compounds that are related to the bacteriocin family (Jack et al., 1995; Klaenhammer, 1993), others are well-known metabolic end products of lactic acid fermentation, lactic, acetic acids and hydrogen peroxide (Vandenbergh, 1993) and certain compounds are unidentified (Bernet Camard et al., 1997; Coconnier et al., 1997; Silva et al., 1987). In worldwide emergence the bacterial resistance to antimicrobial agents has produced a need of new methods for eradicating bacterial infection. This need is forced on researchers by a long time in developing alternative therapy to antimicrobial agents (Huouinen, 2001). Antimicrobial agents reduce the quality of the life because of its diminishing effectiveness due to increase in development of microbial resistance and there by it also disturbs the balance of body by killing friendly bacteria (Reid and Bruce, 2003). These antagonistic substances produced by LAB are termed as bacteriocins, have been reported to inhibit a wide range of Gram positive and Gram negative bacteria as well as fungi (Ocana and Elena Nader-Macias, 2004).
Bacteriocin are highly specific antibacterial proteins produced by the strains of bacteria, which are active mainly against some other strains of same or related species (Gaur et al., 2004) characterized by lethal biosynthesis, interspecific activity and adsorption to specific receptors (Tagg et al., 1976). The bacteriocin produced by LAB has been known for many years (Klaenhammer, 1993) are potent bioactive preservative agents and the applications of these in food are currently the subject of extensive research. The high potential of LAB to produce bacteriocin implies the ability of different strains to produce identical bacteriocin rather than the existence of an almost unlimited number of bacteriocin still not identified.

Several studies on plasmid associated bacteriocin production in lactic acid bacteria have been reported (Ray et al., 1989; Mortvedt and Nes, 1990). It is well known that the association between plasmids and bacteriocin production is generally determined by the use of chemical curing agents and others which eliminate plasmids in the tested strains (Bringel et al., 1989; Floriano et al., 1998).

*Lactobacillus* plays a major role in dairy industries for producing sweeteners. Mannitol is an osmotic diuretic agent and Sorbitol also known as glucitol-sugar alcohol of low calorie is at present gaining importance as sweeteners industrially.

Mannitol is used as a sweetener for people with diabetes, since it has a negative heat solution, it is used as a sweetener in breathe freshening candies, the cooling effect adding to the fresh feel. Mannitol is produced by sourdough lactobacilli from fructose with concomitant formation of acetate. Though sorbitol having the same function as that of mannitol, one advantage of this sweetener is that it can maintain the diet conditions in diabetes.

Sorbitol is naturally produced by human body, is largely used as an ingredient on its sweetening property and high solubility nature. It is naturally found in many fruits (especially apples) (Budavari et al., 1996). Sorbitol is resistant to metabolism by oral bacteria which breaks down sugars and starches to release acids that may lead to cavities or corrode tooth enamel. They are therefore non-carcinogenic. Sorbitol is slowly absorbed and therefore, when it is used, the raise in blood glucose and the insulin response associated with ingestion of glucose is significantly reduced. The reduced caloric value of sorbitol results in weight control. Sorbitol is also a raw
material for production of vitamin C. Sorbitol with such higher upgrading nature produced by a beneficial organism like lactobacilli can be suggested as good bacteria for therapies and production of vaccines. It is also produced by *Lactobacillus* from fructose-6-phosphate with aid of sorbitol phosphate dehydrogenase genes. Metabolic engineering approach has been applied to achieve high-level sorbitol production. It is widely used in a range of food products such as confectionery, chewing gum, candy, deserts, ice cream and diabetic foods. It fulfills the role not only as a sweetener but also as a humectant, a texturizer and a softener (Elvers *et al.*, 1994; Silviera and Jonas, 2002).

Fruits, vegetables, animal faeces, milk samples, yogurts etc., are the different sources for isolating LAB. Among this, milk afford as a superior source for isolating LAB having antagonistic and probiotic works. Milk plays a major role as a nutritional source, economically significant because larger quantity of milk are being processed day to day in fermented food product running industries. Milk and its dairy products have found to have conjugated linoleic acid which provides several health benefits including prevention of antherosclerosis, different types of cancer and hypertension and improved immune function (Clement *et al.*, 1994; Kritchevsky, 2000).

Animal milk is known to have been used first as human food during the secondary products revolution around 5000 BC. Dairy and dairy products from animals have the food value same for atleast one year of the animal killed for meat (McGee and Harold, 2004). Cattle milk are used as the source in this study as they are commercially recommended as exceedingly nutritious and consumed supplementary by humans. Each cattle milk has its own advantages over them under utilization. Buffalo milk is a good source of nutrients such as calcium and other nutrients. Similarly cow milk is a good source of calcium and vitamin D and goat milk is easily digested because of smaller fat size and tolerated with lactose intolerant. LAB is widely distributed in the nature and indigenous micro flora in raw milk that plays an important role in humans and animals including the effect on the immune system (Gabriela Perdigon *et al.*, 2001), many food and feed fermentations. Though many beneficial microorganisms are present LAB are choosen as they are probiotic improving some biological functions in the host through different mechanisms by sending signals to active immune cells. Supplementation of such live microorganisms
repairs the deficiencies in the immune system by stimulating the immune response developing the host to infection resistance. LAB is involved in repairing the deficiency rather than addition of foreign chemicals to the body which may have toxic consequences. The metabolites produced by LAB are used as a biopreservative to preserve the food materials by controlling the growth of the pathogenic and spoilage microorganisms (Sullivan et al., 2002).

The contribution of LAB and their role in numerous functions as probiotics in various fields form the basis for this study. Therefore the aim of the present work is to characterize *Lactobacillus* producing bacteriocin from cattle milk samples, to investigate their antimicrobial and antibiotic activity, assay of bacteriocin, characterization of the bacteriocin produced with respect to physical and chemical parameters and cultural conditions on bacteriocin production, *in vitro* adhesion of the bacteriocin producers to uro-epithelial cells and their antagonism activity against *E. coli*, detection of the size of the bacteriocin protein from the isolates (Tricine SDS-PAGE), *in vitro* study on plasmid DNA oriented or chromosomal DNA oriented bacteriocin production, 16S rRNA sequencing of *Lactobacillus* carrying sweetening gene and screening of sorbitol producing sweetening gene.