2. REVIEW OF LITERATURE

The preservation of foods in healthy and safe condition has long been used and still it remains an on-going challenge for food microbiologists. Drying, salting and fermentations were the traditional methods of preservation. Canning and freezing were relatively recent developments.

The role of fermented milk in human diet is well known since Vedic times but the scientific interest arose only after the publication of a book “Prolongation of Life” (Metchnikoff, 1908). In developed societies, food preservation is viewed as a ‘convenience’ of an efficient food system, and food preservation is the key to ensure the availability of food as vital benefit. Food fermentations, developed by default rather than by design. Lactic acid bacteria (LAB) play an important role in food fermentations, causing the characteristic flavor, changes and exercising a preservation effect on the fermented product (Caplice and Fitzgerald, 1999; Kabuki et al., 2006; Sharma et al., 2006). It is estimated that 25% of the European diet and 60% of the diet in many developing countries consists of fermented foods (Holzapfel et al., 1995). The spice trade was the start of addition of the chemicals adjunct to foods. With the industrial revolution and subsequent development of food industries, food processing moved from kitchen or cottage industries to large-scale technological operations with increased need for food preservation. This stimulated the use of food additives, especially those that preserve the foods and enhance food quality. In recent years the addition of chemical preservatives has fallen into disfavor with consumers, who it is claimed, are seeking for the right quality, less severely processed (less intensive heating and minimal freezing damage), less heavily preserved, more natural (free from artificial additives) and safer (Goud, 1992). This has resulted in the emergence of a new generation of chill stored, minimally processed foods (Oyetayo, 2004; Sharma and Garg, 2005; de Souza et al., 2005).

Hurst (1973) reviewed the preservation of foods by the antagonistic growth of microorganisms. He showed the growth of lactic acid bacteria (LAB)
in milk, saurkaut and vacuum packaged meats as examples of protective and antagonistic growth. In recent times this has been termed as ‘biopreservation’ to differentiate it from the chemical (artificial) preservation of foods.

Biopreservatives such as lactic acid bacteria (LAB) and their metabolites have been investigated by several authors (Buncic et al., 1997; Pirttijarvi et al., 2001; Sakhare and Narasimha Rao, 2003; Sharma and Garg, 2005; Sharma et al., 2006; Kabuki et al., 2006). Considerable research has been done on the ability of LAB to inhibit growth of pathogenic microorganisms (Winkowski et al., 1993; Minor-Perez et al., 2004; Sharma and Garg, 2005; Sharma et al., 2006). The capability of these bacteria to control growth of spoilage microorganisms has not been investigated to the same extent. To be successful in biopreservation, a bacteriocinogenic LAB culture must compete with the relatively high indigenous microbial load of raw meat, to actively inhibit pathogenic and spoilage bacteria (Sakhare and Narasimha Rao, 2003; Minor-Perez et al., 2004; Savadogo et al., 2006).

Bacteria preserve foods as a result of competitive growth, products of their metabolism and bacteriocin production. Biopreservation refers to extended storage life and enhanced safety of foods using their natural or controlled microflora and (or) their antibacterial products. It may consist of (i) adding bacterial strains that grow rapidly and (or) produce their antibacterial products; (ii) adding purified antagonistic substance(s); (iii) adding the fermentation liquor or concentrate from an antagonist microorganism; or (iv) adding mesophilic LAB and other related bacteria as a ‘fail-safe’ protection against temperature abuse.

LAB and other related bacteria produce lactic acid or lactic and acetic acid, and they may produce other inhibitory substances such as diacetyl, hydrogen peroxide, reuterin (β-hydroxypropionaldehyde) and bacteriocins (de Vuyst and Degeest, 1999; Rodrigues et al., 2003; Sharma and Garg, 2005; Sharma et al., 2006).
2.1 Bacteriocins:- Bacteriocins have been described as ribosomally synthesized extracellular macromolecular precursor polypeptides or proteins produced by one bacterium that are active against other bacteria, either in the same species (narrow spectrum), or across genera (broad spectrum) and, as with host defence peptides (Jack et al., 1995; Russell and Mantovani, 2002; Bowdish et al., 2005). Bacteriocins have bacteriocidal activity (Tagg et al., 1976) due to the combined action of the bacteriocin and the host autolysis (Martinez-Cuesta et al., 2000), or bacteriostatic against other species, usually closely related to the producer strain (Russell and Mantovani, 2002). In some cases, they are also active against other species (Klaenhammer, 1993; Jack et al., 1995).

Bacteriocins are heterogeneous group of bacterial antagonists that vary considerably in molecular weight, biochemical properties, range of sensitive hosts and mode of action. Klaenhammer (1988) defined them as, proteins or protein complexes with bactericidal activity directed against species that are usually closely related to the producer bacterium. As peptides, bacteriocins are of low molecular weight, but larger than antibiotics. This makes them susceptible to biochemical reactions, which may limit their antimicrobial activity (Muriana, 1996). CAST (Council of Agricultural Science and Technology) (1998) reported that concentration, microorganisms, pH, temperature and interactions affect the activity of bacteriocins.

Bacteriocins are produced by both Gram-positive and Gram-negative bacteria including LAB, which are used in food fermentations (Klaenhammer, 1988; Daeschel, 1989; Hoover, 1992; Ray and Daeschel, 1992; Hoover and Steenson, 1993; Nettles and Barefoot, 1993; Klaenhammer, 1993; de Vuyst and Vandamme, 1994a; Dodd and Gasson, 1994 Stiles, 1994; Sahl et al., 1995; Muriana, 1996; Pathak et al., 1998; Soomro et al., 2002; Sharma and Garg, 2005; Sharma et al., 2006; Savadogo et al., 2006) Two well-known representatives of bacteriocins produced by Gram-negative bacteria are colicins and microcins. The first description of bacteriocin-mediated inhibition was reported 80 years ago, when antagonism between strains of Escherichia
coli was first discovered (Gratia 1925), they were named as colicins (Fredericq, 1948). Bacteriocins produced by *Escherichia coli* and usually showing activity against other strains of *E. coli* and very closely related members of the Enterobacteriaceae. Induction usually occurs under stressful conditions such as nutrients depletion or over crowding (Riley and Gordon 1999). Colicins have been studied for over six decades and are well characterized (Akutsu et al., 1989; James et al., 1992; James et al., 1996; Pagie and Hogeweg, 1999). Colicins differ from bacteriocins that are produced from Gram-positive bacteria in the sense that they have 3 general mechanisms of action: channel formation in the cytoplasmic membrane, (The common mechanism found with Gram-positive bacteriocins), degradation of cellular DNA, and inhibition of protein synthesis. It is estimated that about 30% of natural population of *E. coli* produce bacteriocins (Riley, 1998). Colicins are plasmid encoded bacteriocins and classified into groups on the basis of the receptor to which they bind. Over 25 colicin types have been identified (Pugsley, 1984). It is also estimated that about 65% of the cells in a population of *E. coli* are resistant to any one colicin, and 30% are resistant to all colicins produced in a population with the remaining cells colicin-sensitive (Smarda, 1992). The relative numbers of colicin-producing cells have been found dependent on the energy costs associated with colicin synthesis (Riley and Gordon, 1999).

The Gram-negative bacteriocins are colicin, which are produced by strain of *E. coli* (Braun et al., 1994; Gordon and Riley, 1999). These are large, complex proteins, that inhibit bacterial growth through the inhibition of cell synthesis, permeabilizing the cell membrane or inhibiting Rnase or Dnase activity (Cleveland et al., 2001) 20-90 Kda, with characteristic structural domains involved in cell attachment, translocation and bactericidal activity. They bind to specific receptors on the outer membrane of the target cell. The bacteriocins produced by Gram-positive bacteria are small peptides 3-6 Kda, in size (Nes et al., 1996), although there are exceptions (Joerger and Klaenhammer, 1990). They fall with in two broad classes, viz (namely) the
lantibiotics (Jack et al., 1995) and the non-lantibiotic bacteriocins (Nes et al., 1996). Most of the Gram-positive bacteriocins are membrane active compounds that increase the permeability of the cytoplasmic membrane (Jack et al., 1995). They often show a much broader spectrum of bactericidal activity than the colicins. There is currently much interest in the application of bacteriocins in both food preservation and the inhibition of pathogenic bacteria (Liao et al., 1994; Yang and Ray, 1994a; Delves-Broughton, 1996; Cleveland et al., 2001; Oyetayo, 2004; de Souza et al., 2005). Most of the bacteriocins have been isolated from organisms involved in food fermentation. Bacteriocin production and resistance is considered as an important property in strains used as commercial inoculants to eliminate or reduce growth of undesirable or pathogenic organisms.

Microcins, produced by the Gram-negative bacteria of family Enterobacterioaceae, are post-translationally modified. They are active against other Gram-negative bacteria and act through inhibition of DNA replication or protein synthesis (Bacquero and Moreno, 1984; Yorgey et al., 1992).

2.2 Lactic acid bacteria (LAB):- Lactic acid bacteria (LAB) have played a long and important role in food technology. These microorganisms are industrially important and have been used as starter cultures in various foods-fermentation processes. Global production of cheese starter cultures, for example, already 1.5 x 10^6 tons per year (Fox, 2002). The LAB include a wide variety of cell types and physiological and biochemical characteristics (Yanagida et al., 2005). Lactic acid bacteria (LAB) are a group of bacteria united by a constellation of morphological, metabolic and physiological characteristics. The currently recognized genera of LAB are Aerococcus, Alloicoccus, Carnobacterium, Dolosigranulum, Enterococcus, Globicalella, Lactococcus, Lactosphaera, Leuconostoc, Melissiococcus, Oenococcus, Pediococcus, Streptococcus, Tetragenococcus, Vagococcus and Weissella (Axelsson, 1990; Ercolini et al., 2001, Sharma et al., 2006). This classification is largely based on phenotypic characteristics such as morphology, mode of
glucose fermentation, growth at different temperature, configuration of lactic acid produced, ability to grow at high salt concentration, and acid or alkaline tolerance (Axelsson, 1998).

Generally, LAB are described as Gram-positive, non-motile, non-spore forming and microaerophilic rods (singly or in chains) or cocci (diplococci, tetracocci, streptococci) (Franke and Buchto, 1960). These bacteria usually belong to the family Lactobacteriaceae and are characterized by the production of lactic acid as a major metabolic end product of carbohydrate fermentation (Axelsson, 1998; de Vos and Hugenholtz, 2004), hydrogen peroxide, diacetyl secondary reaction products and bacteriocins, which may be important for starter culture functions of the bacteria (Daeschel, 1989). The characteristics of LAB used as a starter culture are well documented (Tramer and Fowler, 1964; Carminati et al., 1989; Gupta and Batish, 1990; Davitson and Hoover, 1993; Akcelik et al., 1996; Durlu-Ozkaya 2001; Durlu-Ozkaya et al., 2001). Lactic acid bacteria (LAB) are low-GC-content, Gram-positive bacteria, which are found in nutrient-rich environments such as milk, meat, decomposing plant material and the mammalian gastrointestinal tract, (Carr et al., 2002). Surface growth on most media is very poor. The nutritional requirement of this group is complex; they need amino acids and vitamins.

Lactic acid bacteria may be homofermentative or heterofermentative. Those bacteria which ferment only lactic acid from lactose are known as homofermentative and those that ferment other than lactic acid, e.g., acetic acid alcohol and produce CO₂ are heterofermentative. They are catalase negative, acid tolerant, and lack cytochromes and porphyrins (Adams and Moss 1995).

Lactic acid bacteria (LAB) in the form of fermentative organisms are traditionally used to preserve food and feed. It is well known that many species of Lactobacillus and Lactococcus used in the manufacture of fermented dairy products inhibit the growth of other bacteria including intestinal pathogens like Escherichia coli, Enterobacter faecalis, etc. Bacteriocins produced by LAB are of great interest to the food industry because of their antagonistic effect
against food borne pathogenic and spoilage microorganisms (Matchikoff, 1908; Rogers, 1928; Eckner, 1992; Kone and Fung, 1992; De Vuyst and Vandamme, 1994a; Ray et al., 2001; Guerra and Castro, 2002; Kabuki et al., 2006). The inhibitor part is a protein that could not be destroyed in milk even on heating at 100°C for 30 min and was inhibitory to several strains of Streptococcus lactis. Among the microorganisms inhibited by certain bacteriocins, numerous reports have included the fatal pathogen Listeria monocytogenes (Harris et al., 1989; Spelhang and Harlander, 1989; Muriana, 1996; Klaenhammer, 1993; Ennahar et al., 2000b; Hechard and sahl, 2002; Viveka et al., 2004; Mauriello et al., 2004).


2.3 Taxonomy of lactic acid bacteria:- The classification of LAB was initiated in 1919 by Orla Jensen and was until recently primarily based on morphological, metabolic and physiological criteria. Lactic acid bacteria comprise a diverse group of Gram positive, non-spore forming, non-motile rod and coccus shaped, catalase-lacking organisms. They are chemoorganotrophic and only grow in complex media. Fermentable carbohydrates and higher alcohols are used as the energy source to form chiefly lactic acid. LAB degrades hexoses to lactate (homofermentatives) or lactate and additional products such as acetate, ethanol, CO₂, formate or succinate (heterofermentatives). They are widely distributed in different ecosystems and are commonly found in foods (dairy products, fermented meats and vegetables, sourdough, silage, beverages), sewage, on plants but
also in the genital, intestinal and respiratory tracts of man and animals whose they play important roles as symbionts.

Current methodologies used for classification of LAB mainly rely on 16S ribosomal ribonucleic acid (rRNA) analysis and sequencing (Olsen et al., 1994). Based on these techniques, Gram-positive bacteria are divided into two groups depending on their G + C content. The Actinomycetes have a G + C content above 50 mol% and contain genera such as Atopobium, Bifidobacterium, Corynebacterium and Propionibacterium. In contrast, the Clostridium branch has a G + C content below 50 mol% and include the typical LAB genera Camobacterium, Lactobacillus, Lactococcus, Leuconostoc, Pediococcus and Streptococcus.

Table 1: Orla-Jensen (1919) key to differentiation of the lactic acid bacteria and current taxonomic classification.

<table>
<thead>
<tr>
<th>Genus*</th>
<th>Shape</th>
<th>Catalase</th>
<th>Nitrite reduction</th>
<th>Fermentation</th>
<th>Current genera</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beijer bacterium</td>
<td>Rod</td>
<td>-</td>
<td>-</td>
<td>Hetero</td>
<td>Lactobacillus Weissella</td>
</tr>
<tr>
<td>Thermobacterium</td>
<td>Rod</td>
<td>-</td>
<td>-</td>
<td>Homo</td>
<td>Lactobacillus</td>
</tr>
<tr>
<td>Streptobacterium</td>
<td>Rod</td>
<td>-</td>
<td>-</td>
<td>Homo</td>
<td>Lactobacillus</td>
</tr>
<tr>
<td>Streptococcus</td>
<td>Coccus</td>
<td>-</td>
<td>-</td>
<td>Homo</td>
<td>Camobacterium</td>
</tr>
<tr>
<td>Streptococcus</td>
<td>Coccus</td>
<td>-</td>
<td>-</td>
<td>Homo</td>
<td>Enterococcus</td>
</tr>
<tr>
<td>Leuconostoc Oenococcus</td>
<td>Coccus</td>
<td>-</td>
<td>-</td>
<td>Hetero</td>
<td>Weissella</td>
</tr>
<tr>
<td>Microbacterium</td>
<td>Rod</td>
<td>+</td>
<td>+</td>
<td>Homo</td>
<td>Brochothrix</td>
</tr>
<tr>
<td>Tetracoccus</td>
<td>Coccus</td>
<td>+b</td>
<td>+</td>
<td>Homo</td>
<td>Pediococcus</td>
</tr>
</tbody>
</table>

*According to Orla-Jensen (1919).

**In genera Pediococci are catalase negative but some strains produce a pseudocatalase that results in false positive reactions.

+=Positive result, -=Negative result
2.4 Bacteriocins from lactic acid bacteria:- The bacteriocins from LAB are mostly small, heat-stable, hydrophobic and cationic peptides (Jack et al., 1995). Several bacteriocins of LAB have been characterized biochemically and genetically and in a number of cases their mode of action has been studied (De Vuyst, 1993; Hoover and Steenson, 1993; Klaenhammer, 1993; Kok et al. 1993; Keren et al. 2004; Lima and Filho, 2005; Sharma and Garg, 2005; Chen and Yanagida, 2006; Kabuki et al. 2006; Savadogo et al., 2006). Ever since, the publication of the first review on the bacteriocins of Gram-positive bacteria by Tagg et al. (1976), there has been a renewed interest in the field of bacteriocins of Gram-positive bacteria. The researches on bacteriocins produced by a heterogeneous group of Gram positive bacteria comprising genera, Lactobacillus, Lactococcus, Leuconostoc, Pediococcus, Streptococcus and Camobacterium, collectively known as lactic acid bacteria (LAB) has witnessed tremendous growth in the past one and half decade as evident from the publications of several review articles (Klaenhammer, 1988; Piard and Desmazeaud, 1992; Klaenhammer, 1993; Nettles and Barefoot, 1993; Malik et al., 1994b; Jack et al., 1995; Stiles, 1996b) and books (Ray and Daeschel, 1992; Hoover and Steenson, 1993; Devuyst and Vandamme, 1994b) dealing with various aspects of bacteriocins produced by lactic acid bacteria. Interest in microorganisms as a component of biological diversity has been renewed in recent years (Alsopp et al., 1995). The interest in microorganisms occurring in foods is primarily due to the biotechnological potential of new bacterial species and strains (Leisner et al., 1999; Mierav et al. 2005; Pongtharangkul and Demirci, 2006).

In the dairy products, the species composition of lactic acid bacteria is more varying and inconsistent when compared with those of the trade products. In biotechnological aspects, the "wild" strains of the LABs are prospective bacteriocin producers (Niku-paavola et al., 1999; Park et al., 2003) and probiotics (Rinkinen et al., 2003; Sharma, 2002; Hernandez et al. 2005; Sharma et al. 2006; Joshi et al. 2006).
Bacteriocin producing LAB in food preservation has led to the isolation and characterization of several bacteriocins. The bacteriocin producing LAB have been isolated from various sources such as vegetables, meat and meat products, milk and milk products etc. In some cases, an identical bacteriocin may be produced by different subspecies of the same species as observed for lactococcin A (Neve et al., 1984; Holm et al., 1991; Stoddard et al., 1992). There are also incidents where a single strain produces more than one bacteriocin as recorded for L. lactis subsp. cremoris 9B4 (van Belkum et al., 1991a, 1992) and Lactobacillus plantarum LPC010 (Jimenez-Diaz et al., 1993).

Numerous bacteriocins produced by species and strains of LAB were identified in 1980s and 1990s. These include lactocin and helveticin (Lactobacillus helveticus), lactocin B and F (Lactobacillus acidophilus), curvacin (Lactobacillus curvatus), propioncin (Propionibacterium spp.), platanicin A (Lactobacillus plantarum), Las 5 and diplococcin (Streptococcus cremoris), mesenterosins and leuconosins (Leuconostoc spp.) and pediocins (Pediococcus acidilactici and Pediococcus pentasaceous) (Klaenhammer, 1988; Daeschel, 1989; Hoover and Steenson, 1993; Ray and Daeschel, 1994; Hill, 1995; Mc Mullen and Stiles, 1996; CAST, 1998).

2.4.1 Classification of bacteriocin from LAB:- During recent years, a large number of novel bacteriocins have been identified from several different LAB. Based on their amino acid sequences, stability to heat, size, mode of action, biological activities, secretion mechanism and the presence of modified amino acids, LAB bacteriocins have been classified into three classes of which the first two classes have further been subtyped (Jung, 1991a,b; Klaenhammer et al., 1992; Klaenhammer, 1993; de Vyust and Vandamme, 1994; Dodd and Gasson, 1994; Nes et al., 1996).
Figure 1: Lanthionine synthesis. As shown in figure, lanthionine residues are formed when an enzymatically dehydrated serine (dehydroalanine, Dha) condenses with the sulphhydryl group of a neighbouring cysteine (Cys). This forms a bridge between the two residues, thereby creating a ring within the modified peptide or lantibiotic. When the partners are threonine (Thr) and cysteine, the novel residue is a \( \beta \)-methylthioninone. The resulting lanthionine and \( \beta \)-methylthioninone bridges are indicated in pink as Ala–S–Ala (alanine–S–alanine) and Abu–S–Ala (aminobutyrate–S–alanine), respectively. Many lantibiotics also contain dehydrated serines (Ser) and threonines (dehydrobutyric, Dhb).

Source: Cotter et al. (2005).

Class I- Lantibiotics (from lanthionine-containing antibiotic) are small (< 5kDa) peptides containing the unusual amino acids lanthionine (Lan), \( \alpha \)-methylthioninone (melan), dehydroalanine, and dehydrobutyric. These bacteriocins are grouped in class I. Class I is further subdivided into type A and type B lantibiotics according to chemical structures and antimicrobial activities (Moll et al., 1999; Van Kraaij et al., 1999; Guder et al., 2000). Type A lantibiotics are elongated peptides with a net positive charge that exert their activity through the formation of pores in bacterial membranes. Type B lantibiotics are smaller
globular peptides and have a negative or no net charge; antimicrobial activity is related to the inhibition of specific enzymes. They are heat stable protein. e.g. nisin, lacticin 481, lactocin 5, Carnocin U 149 etc.

**Class II**- Small (< 10kDa), heat-stable, non-lanthionine containing peptides are contained in class II. The largest group of bacteriocins has been included in this classification system. These peptides are divided into 3 subgroups.

**Class IIA** includes pediocin-like peptides having N-terminal consensus sequence –Tyr-Gly-Asn-Gly-Val-Xaa-Cys. This subgroup has attracted much of the attention due to their anti-listeria activity (Ennahar et al., 2000b).

**Class IIB** contains bacteriocins requiring two different peptides for activity, e.g. lactococcin G and lactocin F.

**Class IIC** contains the remaining peptides of this class, including seq-dependent secreted bacteriocins (Worobo et al., 1995). e.g. divergicin A.

**Class III**- These bacteriocins are not well characterized. This group contains large (> 30kDa) heat-labile proteins that are of lesser interest to food scientists. (Joeger and Klaenhammer, 1986; Vaughan et al., 1992; Thompson et al., 1996) e.g., helveticin I, caseicin 80, lacticins A and B.

A class IVth class consisting of complex bacteriocins that require carbohydrate or lipid moieties for activities has also been suggested by Klaenhammer (1993); however, bacteriocins in this class have not been characterized adequately at the biochemical level to the extent that the definition of this class requires additional descriptive information (Jimenez-Diaz et al., 1995; Mc Auliffe et al., 2001).
Cotter et al. (2006) have proposed a new scheme of classification for bacteriocins, which is reproduced below in Figure 2.

**Figure 2: Proposed classification scheme for bacteriocins.**

Source: Cotter et al. (2006)

Chen and Hoover (2003) have summarized different classes of bacteriocins and their producer strain, which are reproduced below in Table 2.

**Table 2: Examples of bacteriocin producing by lactic acid bacteria:**

<table>
<thead>
<tr>
<th>BACTERIOCINS</th>
<th>PRODUCER</th>
<th>REFERENCES</th>
</tr>
</thead>
<tbody>
<tr>
<td>CLASS I-type A lantibiotics</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nisin</td>
<td><em>Lactococcus lactis</em></td>
<td>Hurst 1981</td>
</tr>
<tr>
<td>lactocin S</td>
<td><em>Lactobacillus sake</em></td>
<td>Mortvedt et al., 1991</td>
</tr>
<tr>
<td>Epidermin</td>
<td><em>Staphylococcus epidermidis</em></td>
<td>Allgaier et al., 1986</td>
</tr>
</tbody>
</table>

Table 2-Continued
<table>
<thead>
<tr>
<th>Class</th>
<th>Lantibiotic</th>
<th>Producing Microorganism</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Class I-typed Lantibiotics</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>lacticin</td>
<td>Lactococcus lactis</td>
<td>Piard et al., 1992</td>
<td></td>
</tr>
<tr>
<td>CLASS I-typed Lantibiotics</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mersacidin</td>
<td>Bacillus subtilis</td>
<td>Altena et al., 2000</td>
<td></td>
</tr>
<tr>
<td>cinnamycin</td>
<td>Streptomyces cinnamoneus</td>
<td>Sahl and Bierbaum 1998</td>
<td></td>
</tr>
<tr>
<td>ancovenin</td>
<td>Streptomyces spp.</td>
<td>Sahl and Bierbaum 1998</td>
<td></td>
</tr>
<tr>
<td>duramycin</td>
<td>Streptomyces cinnamoneus</td>
<td>Sahl and Bierbaum 1998</td>
<td></td>
</tr>
<tr>
<td>actagardin</td>
<td>Actinoplanes spp.</td>
<td>Sahl and Bierbaum 1998</td>
<td></td>
</tr>
<tr>
<td>CLASS IIa</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pediocin PA-1/AcH</td>
<td>Pediococcus acidilactici</td>
<td>Henderson et al., 1992; Morlagh et al., 1992</td>
<td></td>
</tr>
<tr>
<td>sakacin A</td>
<td>Lactobacillus sake</td>
<td>Holck et al., 1992</td>
<td></td>
</tr>
<tr>
<td>sakacin P</td>
<td>Lactobacillus sake</td>
<td>Tichaczek et al., 1992</td>
<td></td>
</tr>
<tr>
<td>leucocin A-UAL187</td>
<td>Leuconostoc gelidum</td>
<td>Hastings et al., 1991</td>
<td></td>
</tr>
<tr>
<td>Mesentericin Y105</td>
<td>Leuconostoc mesenteroides</td>
<td>Hechard et al., 1992</td>
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<tr>
<td>enterocin A</td>
<td>Enterococcus faecium</td>
<td>Aymerich et al., 1996</td>
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<tr>
<td>divercin V41</td>
<td>Carnobacterium divergens</td>
<td>Metivier et al., 1998</td>
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<tr>
<td>lactococcin MMFII</td>
<td>Lactococcus lactis</td>
<td>Ferchichi et al., 2001</td>
<td></td>
</tr>
<tr>
<td>CLASS IIb</td>
<td></td>
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</tr>
<tr>
<td>lactococcin G</td>
<td>Lactococcus lactis</td>
<td>Nissen-Meyer et al., 1992</td>
<td></td>
</tr>
<tr>
<td>lactococcin M</td>
<td>Lactococcus lactis</td>
<td>van Belkum et al., 1991</td>
<td></td>
</tr>
<tr>
<td>lactacin F</td>
<td>Lactobacillus johnsonii</td>
<td>Allison et al., 1994</td>
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<td>plantaricin A</td>
<td>Lactobacillus plantarum</td>
<td>Nissen-Meyer et al., 1993a</td>
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<tr>
<td>plantaricin S</td>
<td>L. plantarum</td>
<td>Jimenez-Diaz et al., 1995</td>
<td></td>
</tr>
<tr>
<td>plantaricin EF</td>
<td>L. plantarum</td>
<td>Anderssen et al., 1998</td>
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Table 2-Continued
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<thead>
<tr>
<th>Plantaricin JK</th>
<th>L. plantarum</th>
<th>Anderssen et al., 1998</th>
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<tr>
<td><strong>CLASS IIc</strong></td>
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</tr>
<tr>
<td>acidocin B</td>
<td>Lactobacillus acidophilus</td>
<td>Leer et al., 1995</td>
</tr>
<tr>
<td>carnobacteriocin A</td>
<td>Carnobacterium piscicola</td>
<td>Worobo et al., 1994</td>
</tr>
<tr>
<td>divergicin A</td>
<td>C. degerens</td>
<td>Worobo et al., 1995</td>
</tr>
<tr>
<td>enterocin P</td>
<td>E. faecium</td>
<td>Cintas et al., 1997</td>
</tr>
<tr>
<td>enterocin B</td>
<td>E. faecium</td>
<td>Nes and Holo 2000</td>
</tr>
<tr>
<td><strong>CLASS III</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>helveticin J</td>
<td>Lactobacillus helveticus</td>
<td>Joergen and Klaenhammer 1986</td>
</tr>
<tr>
<td>helveticin V-1829</td>
<td>L. helveticus</td>
<td>Vaughan et al., 1992</td>
</tr>
</tbody>
</table>

2.4.2 Bacteriocin produced by different group of lactic acid bacteria:

2.4.2.1 *Lactococcus*: - *Lactococci* are coccibacteria, which form chains of variable length. They have a homo-fermentative metabolism and produce exclusively L (+) lactic acid (Roissart, 1994), although Akerberg et al. (1998) reported that, D (-) lactic acid can also be produced specially at low pH values. Furthermore, *Lactococcus lactis* is sub-divided into other sub-species: lactis, cremoris and diacetylactis.(Schleifer and Kilpper-Balz, 1987; Kim et al., 1999). Their most important habitat is untreated milk, fermented milk and cheeses. *Lactococcus lactis* subsp. lactis, either in pure form or associated with other microorganisms, is mesophilic strain most commonly used as a starter culture for lactic products, thus, they fulfill an irreplaceable role in ensuring the structure, taste, conservation and healthfulness of these products. (Jenson and Hammer, 1993; Salminen and Von wright, 1993; Roissart, 1994; Boonmee et al., 2003; Ziadi et al., 2005; Do-won et al., 2006).

They also play an important role in aroma enhancement, the production of flavoured milk, and in milk and cheese flavouring, and recently a great deal of attention has been focused on their probiotic properties (Salminen and Von wright, 1993; Van Niel and Hahn- Hagerdal, 1999; Boonmee et al., 2003). For these reason this microorganism, has great commercial potential, and that is
why Lactococcus, and more especially Lactococcus lactis, isolated in the lactic industry, is still being studied exhaustively. The major product of fermentation is lactic acid, a compound with a high commercial value, with applications in the food, cosmetic, medical and pharmaceutical industries (Boonmee et al., 2003), however, the most interesting application for the lactic acid is its potential in the production of biopolymers (Hujanen and Linko, 1996; Chang et al., 1999; Hujanen et al., 2001; Danner et al., 2002; Lee, 2005).

Several scientists (Oxford, 1944; Mattick and Hirsch, 1947; Neve et al., 1984; Holo et al., 1991 and Kojik et al., 1991) have reported bacteriocinogenicity among the different strains of the three most economically important lactococcal species: Lactococcus lactis subsp. lactis, Lactococcus lactis subsp. cremoris and Lactococcus lactis subsp. lactis biovar. diacetylactis.

2.4.2.1.1 Lactococcus lactis subsp. lactis: -

Nisin: The most extensively characterized bacteriocin from lactic acid bacteria is produced by several strains of Lactococcus lactis subsp. lactis. Mattick and Hirsch coined the word 'nisin' to designate the group 'N' inhibitory substance in 1947. Nisin, is widely used bacteriocin, is normally ineffective against Gram negative bacteria, yeast and moulds, but effective against a wide range of Gram positive bacteria including other lactic acid bacteria, Staphylococcus aureus and Listeria monocytogenes. Gram-positive spore formers i.e. Bacillus spp. and Clostridium spp. are particularly sensitive to nisin with spores being more sensitive than vegetative cells (Ray, 1992; Delves-Broughton et al., 1996).

First elucidated by Gross and Morell in 1971, nisin is a 34 amino acid peptide. At least 6 different forms have been discovered and characterized (designated as A through E and Z), with nisin A, the most active type. Nisin Z is a natural variant of nisin differing from nisin A with substitution of a histidine residue for an aspartic acid. The most established commercially available form of nisin for use as, a food preservative is Nisaplin™, with the active ingredient 2.5% nisin A and the predominates ingredients NaCl (77.5%) and nonfat dry
called 'lantibiotics' a family of peptides containing β unsaturated amino acids, dehydroalanine and dehydrobutyrylne and thio-ether amino acids, lanthionine and β-methyl lanthionine (Gross and Morell, 1967, 1970). It is a protein having 34 amino acids and molecular weight of 3.5 KDa (Klaenhammer, 1988). Nisin has five polypeptide variants, which are designated as A, B, C, D, and E. International acceptance of nisin was given in 1969 by the Joint Food and Agriculture Organization/World Health Organization (FAO/WHO) Expert Committee on Food Additives (WHO 1969). The only other antibiotic-like compound with similar approval as a preservative is the surface-active antimycotic compound, pimaricin (Henning et al., 1986). FAO/WHO Committee recommended a maximum daily intake of nisin for a 70-kg person to be 60 mg of pure nisin or 33000 Units (Hurst and Hoover 1993); however, nisin is permitted in processed cheeses in Australia, France, and Great Britain with no maximum limit. In the U.S., the maximum limit is 10000 IU/g; in Russia, the maximum limit is 8000 IU/g, while in Argentina, Italy, and Mexico, the limit is 500 IU/g for processed cheeses and other products (Chikinda and Montville 2002).

![Figure 3: Milestones in the commercial development of nisin](image)

**Source:** Cotter, et al. (2005).

In the USA, the FDA has affirmed a nisin preparation as a GRAS (Generally recognized as safe) substance for use in pasteurized cheese spreads for inhibition of outgrowth of *Clostridium botulinum* (CFR, 1996). Shhtenberg and Ignatev already reported the toxicity of nisin in 1970. They
spreads for inhibition of outgrowth of Clostridium botulinum (CFR, 1996). Shntenberg and Ignatev already reported the toxicity of nisin in 1970. They suggested that the toxicity of nisin is low and it is not used in animal or human medicine.

A bacteriocin, lactacin 481, produced by Lactococcus lactis subsp. lactis CNRZ481 was found to be effective against Lactococcus spp., some Lactobacillus spp., Leuconostoc spp., and Clostridium spp. reported by Piard et al. (1990) The production of several lactococcins has been described in several other Lactococcus lactis subsp. lactis which include; Lactococcin by Lactococcus lactis subsp. lactis ADRI 85L030 (Dufour et al., 1991) has been found to inhibit vegetative cells of Clostridium tyrobutyricum, strains of Streptococcus thermophilus and Lactobacillus helveticus but is rather inactive against other Gram positive and Gram negative genera (Thuault et al., 1991); lactococcin G by Lactococcus lactis subsp. lactis LMG2081 (Nissen-Meyer et al., 1992); lactococcin 972 by Lactococcus lactis subsp. lactis (Martinez et al., 1996); lactococcin 484 by Lactococcus lactis subsp. lactis 484 has been reported to be effective against members of the Lactococcus group, B. cereus, Staphylococcus aureus and Salmonella typhi (Gupta and Batish, 1992).

Structure of bacteriocin: - Nisin is well known member of bacteriocin and is produced by strains of Lactococcus lactis subsp. lactis. Its structure is illustrated in Fig-4. (Kaletta and Entian. 1989). The name “nisin” for this bacteriocin is derived from the term “group N inhibitory substance” (where group N refers to sero group N of bacteria classified as member of the genus Lactococcus). Nisin consists of 34 amino acids, however, it is initially synthesized as prenisin, consisting of 23 amino acid leader peptide and the 34- amino acid pronisin peptide. (Nes, et al.1996).
Figure 4: Nisin structure.

Source: Cotter et al. (2005).

Variants of nisin differing in 1 amino acid are known. Certain serine and threonine residues in the pronisin are converted to dehydroalanine and dehydrobutyrine through dehydration. Thioether bonds are then formed by reaction with the sulfhydryl groups of cysteine residues in the pronisin (lanthionine, Ala-S-Ala; β-methyllanthionine; Ala-S-Abu; aminobutyric acid). Following these chemical modifications in the pronisin segment of prenisin, export and concomitant removal of the leader sequence yield active nisin.

Normally, two nisin molecules form a dimer. The early reports on the molecular weight of nisin as 7 kDa (approx.) where, due to the formation of dimers rather than the approximately 3.5 kDa measured only (Joerger and Hoover, 2000), Sharma (2002) also reported 3.5 kDa molecular weight of nisin isolated from Lactococcus lactis subsp. lactis CCSU1101.

Figure 5: Lacticin A1 and A2 structure.

2.4.2.1.2 *Lactococcus lactis* subsp. *cremoris*: - The first description of a proteinaceous inhibitor in lactococci was from *Lactococcus lactis* subsp. *cremoris*. The antimicrobial agent described by Whitehead in 1933 was later on partly purified and shown to be proteinaceous in nature. It was termed as 'Diplococcin' to signify the diplococcal arrangement of the producer cells (Oxford, 1944). A number of lactococcins have been described for *Lactococcus lactis* subsp. *cremoris*. These include: lactococcin A from strain LMG2130 (Holo et al., 1991) and strain 9B4 (Neve et al., 1984). Later it was found that *Lactococcus lactis* subsp. *cremoris* strain 9B4 produced two more bacteriocins termed as lactococcin M (van Belkum et al. 1991a) and lactococcin B (van Belkum et al. 1992). Huot et al (1996) described the production of a bacteriocin designated as Bacteriocin J46 by *Lactococcus lactis* subsp. *cremoris* J46.

Many substances have been reported from *lactococci*, which have been designated as lactococcin. These include lactococcin I, A, B and M, lactococcin I has been isolated from *Lactococcus lactis* subsp. *cremoris* strain A and C. Purified lactococcin has been reported to be heat stable (99°C, 33 min) peptide with a molecular weight of 6,000 Da. It is encoded by a 18.4 Kb fragment of DNA of a 60 Kb conjugative plasmid. It has been reported to inhibit other *Lactococci* and some *Clostridia*. (Geise et al. 1983).

Lactococcin A has been reported to be produced by three different *Lactococci*, which include 1.8 Kb region of plasmid p9 B4-6, from *Lactococcus lactis* subsp. *cremoris* 9B4, associated with production of another bacteriocin. It has also been cloned and analyzed (Van Belkum, et al., 1991a). Nucleotide sequence analysis of the resulting plasmid (pMB225) showed a ribosomal binding site followed by three ORFs designated ORFA-1 ORFA-2 and ORFA-3. The third ORF, ORFA-3 is suspected to encode an immunity protein for lactococcin M (Van Belkum et al. 1991a).
Lactococcin B is a bacteriocin associated with a 1.2 Kb fragment present in plasmid p9B4 of Lactococcus lactis subsp. cremoris 9B4 (Van Belkum et al. 1991a). The genes encoding this bacteriocin are present on the same fragment on which the genes for lactococcin M and lactococcin A are present. Active bacteriocin is a 5300 Da protein.

Diplococcin is one of the earliest bacteriocin isolated from LAB. It is a protein with molecular weight of 5.3 kDa and produced by Lactococcus lactis subsp. cremoris in milk and M17 broth during early stationary phase. It was partially purified by Oxford (1944) and was found to be water-soluble and heat stable under acidic conditions. It differs from nisin in many of its characteristics. It does not contain sulphur containing amino acids, lanthionine and ß-methyl lanthionine, which are the characteristics of lantibiotics (Davey and Richardson, 1981). The inhibitory spectrum of diplococcin from Lactococcus lactis subsp. cremoris was restricted to lactococci only (Davey and Pearce, 1980). Lactococcus lactis subsp. cremoris starin 9B4-secreting lactococcins A, B and M prevented the growth not only of other lactococci but also of some Clostridia (Geis et al. 1983). Holo et al. (1991) purified lactococcin A and found that it inhibited the growth of only lactococci. Out of over 120 strains of different Lactococci tested only one was insensitive to lactococcin A as was the case with all other Gram-positive bacteria. Bacteriocin J46 has a wide spectrum of antibacterial activity including antclostridial activity (Gonzalaz et al. 1996).

Purified diplococcin is unstable at room temperature, rapidly inactivated by heat and degraded by proteolytic enzymes like chymotrypsin, trypsin and pronase (Klaenhammer, 1988). It has a narrow spectrum of activity against closely related Lactococcus lactis and other strains of Lactococcus cremoris (Davey, 1981). It is encoded by 54 Mda plasmid (Davey, 1984). In addition to nisin and diplococcin, lactococci have been reported to produce some other bacteriocins. Hirsch and Grinsted (1951) have reported that streptococci produced a variety of bacteriocins but in comparison to nisin activity of other bacteriocins was much lesser.
2.4.2.1.3 Lactococcus lactis subsp. lactis biovar. diacetylactis: - The bacteriocin described in Lactococcus lactis subsp. lactis biovar. diacetylactis WM4 (Scherwitz et al., 1983) has been found to be identical to the lactococcus A produced by Lactococcus lactis subsp. cremoris strains 9B4 and LMG2030 (Stoddard et al., 1992). Kojic et al. (1991) reported the production of bacteriocin S50 by Lactococcus lactis subsp. lactis biovar. diacetylactis S50. A strain of Lactococcus lactis subsp. lactis biovar. diacetylactis UL720 isolated from raw milk has been found to produce a bacteriocin termed as diacetin B (Ali et al., 1995). Morgan et al (1995) isolated Lactococcus lactis subsp. lactis biovar. diacetylactis DPC398 from an Irish cheese factory and observed the effect of all the three lactococccins viz. A, B, and M in the strain DPC398.

Lactostrepsins are another group of inhibitory substances, which have been reported to be produced by non-nisin producing strains of Lactococcus lactis subsp. diacetylactis, Lactococcus lactis subsp. cremoris and Streptococcus lactis subsp. lactis (Kozak, et al., 1978; Dobrzanski et al., 1982). These substances show maximum activity in the pH range of 4.6 to 5.0, whereas at pH 7.0 the activity is lost (Dobrzanski, 1982). Lactostrepsins are stable at 121°C for 10 minutes and produced in non-agitated broth cultures during early logarithmic phase. These are inactivated by proteolytic enzymes. Their molecular weight exceeds 10,000 Da. These show inhibitory action against other lactococci, group A, C and G streptococci, Bacillus cereus, Lactobacillus helveticus, Leuconostoc mesenteroides subsp. cremoris, and Leuconostoc paracitrovorum. Lactostrepsin 5 produced by Lactococcus lactis subsp. cremoris 202 disrupts the cell membrane, interferes with uridine transport and inhibits DNA, RNA or protein synthesis (Zajdel and Dobrzanski, 1983). Information on genetic determinants responsible for production and immunity is inconclusive.

2.4.2.2 Lactobacilli: - The bacteriocinogenicity has been described for several of the obligate homofermenters (Lactobacillus acidophilus, and Lactobacillus helveticus), facultative heterofermenters (Lactobacillus
plantarum and Lactobacillus sake) and for the heterofermentative Lactobacillus brevis.

2.4.2.2.1 Dairy lactobacilli:

2.4.2.2.1.1 Lactobacillus helveticus: -The bacteriocins described from the species includes helveticin J by the strain Lactobacillus helveticus 481 (Joeger and Klaenhammer, 1986). Helveticin J is sensitive to several proteolytic enzymes and heat and shows its action against limited related Lactobacilli. It is encoded by chromosomal determinants. Crude protein has molecular weight of 30,000 Da but purified protein has a molecular weight of 37,000 Da and helveticin V-1829 by the strain Lactobacillus helveticus 1829 (Vaughan et al., 1992). This bacteriocin has been found to be heat labile (50°C for 30 min.), which is bactericidal against other Lactobacilli. The partially purified preparation is inactivated by proteinase K, trypsin, pronase, heat and pH above 7.0. It is chromosomally encoded and has no plasmids. (Vaughan, et al., 1992). Thompson et al., (1996) identified a bacteriocin in the culture supernatant of Lactobacillus helveticus CNRZ450.

2.4.2.2.1.2 Lactobacillus acidophilus: - Early investigations into the antimicrobial activities of Lactobacillus acidophilus suffered due to insufficient characterization of the antagonistic agents, to determine whether or not bacteriocins are responsible for the observed inhibition (Klaenhammer, 1988). Barefoot and Klaenhammer (1983) provided a more definitive characterization of bacteriocins from the species with the description of lactacin B, a bacteriocin produced by Lactobacillus acidophilus N2. ten-Brink et al. (1994) reported the production of acidocin B, an atypical bacteriocin by Lactobacillus acidophilus strain M46 isolated from human dental plaque and Lactobacillus acidophilus TK9201 was found to produce a bacteriocin termed as acidocin A (Kanatani et al., 1995).

Two bacteriocins, which have been named as lactacin F and B, are produced by Lactobacillus acidophilus 11088 and Lactobacillus acidophilus N2.
plantarum and Lactobacillus sake) and for the heterofermentative Lactobacillus brevis.

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Two bacteriocins, which have been named as lactacin F and B, are produced by Lactobacillus acidophilus 11088 and Lactobacillus acidophilus N2
α amylases. It is interesting to note that *Lactobacillus plantarum* LPC010 isolated from a green olive fermentation elaborated into the growth medium by two bacteriocins designated as plantaricins S and T (Jimenez-Diaz et al., 1993). A bacteriocin, plantaricin KW30, producing strain of *Lactobacillus plantarum* has recently been isolated from fermented maize (Kelly et al., 1996).

Bacteriocinogenic *Lactobacillus plantarum* strains from dairy and meat products have also been reported. A *Lactobacillus plantarum* strain LTF154 isolated from a fermented sausage produced a bacteriocin designated as plantacin 154 (Kanatani and Oshimura, 1994). Rekhif et al. (1994) isolated a bacteriocin producing *Lactobacillus plantarum* strain LC74 from goat raw milk and named the bacteriocin as plantaricin LC74. Recently Ennahar et al. (1996) reported the production of a bacteriocin identical to pediocin AcH by a strain of *Lactobacillus plantarum* WHE92 isolated from a soft cheese.

### 2.4.2.2.2 *Lactobacillus curvatus*: - Curvacin A produced by *Lactobacillus curvatus* LTH 1174, an isolate from meat, produces this bacteriocin which shows inhibitory action against other *Lactobacilli*, *Leuconostoc*, *Comybacteria*, *Listeria monocytogenes*, as well as a weak action against *Micrococcii* and *Staphylococci*. (Tickacze et al. 1992). Like sakacin P, curvacin A is destroyed by proteinase K and trypsin but stable when treated with pepsin or heat (100°C, 3 min). Its molecular weight has been estimated to be 3,000 to 5,000 Da.

### 2.4.2.2.3 *Lactobacillus sake*: - Bacteriocins produced by the strains of *Lactobacillus sake* isolated from meat and fermented sausages include: sakacin A by *Lactobacillus sake* 706 (Schillinger and Lucke, 1989), lactoicin S by *Lactobacillus sake* L45 (Mortvedt and Nes, 1990, 1991; Skaugen et al., 1994).

Sakacins are the bacteriocins produced by *Lactobacillus sake* and include sakacin, A, M, S and P. *Lactobacillus sake* is responsible for
fermentation in sausages. Sakacin A and M inhibit other Lactobacilli as well as Listeria monocytogenes (Schillinger and Lucke, 1989; Schillinger, et al., 1991). Lactocin S inhibits strains of lactic acid bacteria (Mortvedt and Nes, 1990). Sakacin A is produced by Lactobacillus sake 706 and is heat stable (100°C for 20 min.) produced during the mid and late logarithmic growth phase in liquid medium and is associated with a 27.7 Kb plasmid. (Schillinger and Lucke, 1989).

Sakacin M was produced from Lactobacillus sake isolated from Spanish dry fermented sausages. (Sobrino et al. 1991). It is produced maximally in a synthetic medium supplemented with 1.5% tryptone during growth at 32°C. Molecular weight of the bacteriocin has been estimated to be 4640 Da. Inhibitory activity of a partially purified compound is diminished by trypsin, pepsin, papain and protease XIV and II. Crude and partially purified compounds are heat stable at 80°C for 60 minutes and 150°C for 9 minutes. Bacteriocin shows inhibitory action against Lactobacilli, Leuconostoc, Carbonobacteria, Listeria monocytogenes and Staphylococcus aureus (Sorbino et al. 1992).

Another bacteriocin, sakacin P, was produced by Lactobacillus sake LTH 673, isolated from meat and it was found to inhibit Lactobacilli and spoilage organisms like Leuconostoc, Corynbacteria, Enterococci, Brochothrix thermosphacta and Listeria sp. (Tichaczek et al. 1992). Bacteriocin is sensitive to proteinase K and trypsin but insensitive to pepsin and heat at 100°C for 7 minutes. It is a protein of molecular weight 3,000 to 5,000 Da with 36 to 41 amino acid residues.

Lactocin S is a heat stable protein active against Pediococcus, Leuconostoc, and Lactobacilli. It has been isolated from Lactobacillus sake 245. (Mortvedt and Nes, 1990). Molecular weight of crude Lactocin S has been reported to be 30,000 Da, however, partially purified active proteins have molecular weight less than 13,700 Da. Production is associated with an unstable 50 Kb plasmid. (Mortvedt et al., 1991). Mode of action studies
indicated that lactosin S acts bactericidally in a pH dependent fashion (Twomey et al., 2002).

2.4.2.2.4 *Lactobacillus brevis*: Benoit et al. (1994) identified an antibacterial protein produced by *Lactobacillus brevis* SB27 isolated from dry cured sausage and designated it as brevicin 27. Production of brevicin 286 has been recently reported in *Lactobacillus brevis* VB286 that was originally isolated from vacuum packaged meat (Conventry et al., 1996).

*Lactobacillus brevis* produced an antibacterial substance named brevicin 37, which was inhibitory to *Pediococcus* sp., *Leuconostoc* sp., *Lactobacillus* sp. and *Nocardia carolina*. The protein is stable at pH range of 1 to 11. It is also stable to heat at 121°C for 1 h and is retained on a 10,000 molecular weight cut off membrane. (Rammelsberg and Radler, 1990).

2.4.2.2.5 *Lactobacillus casei*: Rammelsberg and Radler (1990) isolated *Lactobacillus casei* B 80 from plants and fermenting materials. *Lactobacillus casei* B 80 produced a heat sensitive protein with a narrow spectrum of activity against other strains of *Lactobacillus casei*. (Rammelsberg et al., 1990). Its molecular weight has been reported to be 40,000 to 42,000 Da.

Other lactobacilli: Lactacin F producing *Lactobacillus acidophilus* 11088 (Muriana and Klaenhammer, 1987) has been renamed as *Lactobacillus johnsonii* as cited by Klaenhammer (1993).

2.4.2.3 *Leuconostoc*: The first evidence for bacteriocin production in *Leuconostoc* spp. was provided by Harding and Shaw in 1990. They reported the production of a heat stable protein by a strain of *Leuconostoc gelidum* that was active against other lactic acid bacteria and three strains of *Listeria monocytogenes*. In recent years, a number of bacteriocin producing strains of *Leuconostoc* species have been isolated from various sources such as milk and meat products.
Hastings and Stiles (1991) reported the production of a bacteriocin-designated leucocin A-UAL187 by *Leuconostoc gelidum* UAL187 isolated from meat packed under elevated (30%) carbon dioxide. *Leuconostoc paramesenteroides* OX isolated by Lewus et al. (1991) from retail lamb was found to produce a bacteriocin named as leuconocin S (Lewus et al., 1992).

Bacteriocins, carnosin 44A, carnocin LA54A and leucocin B-Talla, produced by *Leuconostoc carnosum* LA44A from vacuum packaged Vienna-type-sausage (van Laack et al. 1992), *Leuconostoc carnosum* LA54A from meat (Keppler et al. 1994) and *Leuconostoc carnosum* Talla isolated from vacuum packaged processed meat (Felix et al. 1994), respectively, have been described in the strains of *Leuconostoc carnosum*. Yang and Ray (1994a) observed the predominance of *Leuconostoc carnosum* and *Leuconostoc mesenteroides* in the spoiled low heat processed vacuum packaged meat products. The notable feature of many of these *Leuconostoc* isolates is their ability to produce bacteriocins.

Bacteriocinogenic strains of *Leuconostoc* spp. have also been isolated from milk and milk products. Strains of *Leuconostoc mesenteroides* subsp. *mesenteroides*, Y105 from goat milk and FR52 from raw milk were found to produce bacteriocins, mesentericin Y105 (Hechard et al., 1992) and mesentericin 52 (Mathiew et al. 1993), respectively. Dextranicin J24 was a bacteriocin produced by an isolate of *Leuconostoc mesenteroides* subsp. *dextranicum* J24 from French soft cheese (Sudirman et al., 1994). Malik et al. (1994a) reported the detection and activity of a novel bacteriocin, leucocidin R1, produced by *Leuconostoc paramesenteroides* NM14 isolated from an aged cream sample.

2.4.2.4 Pediococci:-

2.4.2.4.1 *Pediococcus pentosaceus*:- The bacteriocin produced by *Pediococcus pentosaceus* FBB61 from cucumber fermentations was designated as pediocin A (Daeschel and Klaenhammer, 1985). Hoover et al. (1988) observed bacteriocinogenic activity in *Pediococcus pentosaceus* MC03.
isolated from pepperoni, a fermented sausage. Bacteriocin production in *Pediococcus pentosaceus* strain N5p from wine has been reported and the bacteriocin was named as pediocin N5p (Strasser-de-Saad and Manca-de-Nadra, 1993).

### 2.4.2.4.2 *Pediococcus acidilactici*:- The most extensively characterized bacteriocins, pediocin AcH and pediocin PA-1, after nisin have been produced by strains of *Pediococcus acidilactici*. Gonzalez and Kunka, (1987) reported pediocin PA-1 production by *Pediococcus acidilactici* PAC1.0. Pediocin AcH producing *Pediococcus acidilactici* H was isolated by Bhunia *et al.* (1987a) from fermented sausage. Hoover *et al.* (1988) observed the production of unnamed bacteriocins by *Pediococcus acidilactici* PO2 as pediocin PO2. Schved *et al.* (1993) reported the isolation of *Pediococcus acidilactici* SJ1 from a naturally fermented meat product and designated its bacteriocin as pediocin SJ1 while pediocin L50 producing *Pediococcus acidilactici* L50 was obtained from Spanish dry fermented sausage (Cintas *et al.*, 1995).

### 2.4.3 Characteristics of bacteriocins:- Bacteriocins of LAB have been characterized with respect to their (i) sensitivity to various proteolytic and non-proteolytic enzymes (ii) stability to various heat treatments (iii) pH stability (iv) mode of action and (v) molecular weight etc. In most of these characterization studies, either crude or partially purified bacteriocin preparations have been used.

The fact that bacteriocins are proteins renders them sensitive to at least one of the proteolytic enzymes. Apart from protein moiety, some bacteriocins have been found to contain an active lipid or carbohydrate moiety which is also required for antibacterial activity as revealed by loss of bacteriocin activity upon treatment with lipases or amylases (Lewus *et al.*, 1992; van Laack *et al.*, 1992; Jimenez-Diaz *et al.*, 1993; Schved *et al.*, 1993; Keppler *et al.*, 1994)

The term bacteriocin has been restricted to those antibacterial proteins that exhibit a bactericidal mode of action (Tagg *et al.*, 1976). Although, a vast
majority of bacteriocins of LAB exert a bactericidal mode of action, but a few have been found to be bacteriostatic rather than bactericidal to the sensitive cells (Lewus et al., 1992; Thompson et al., 1996). Characteristic of bacteriocin and other conventional antibiotics shown in Table 3.

Table 3: Characteristic aspects of bacteriocins and other conventional antibiotics.

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Characteristics</th>
<th>Bacteriocins</th>
<th>Other antibiotics</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Application</td>
<td>Foods</td>
<td>Clinical</td>
</tr>
<tr>
<td>2</td>
<td>Synthesis</td>
<td>Ribosomally</td>
<td>Secondary metabolism</td>
</tr>
<tr>
<td>3</td>
<td>Activity</td>
<td>Limited spectrum</td>
<td>Wide spectrum</td>
</tr>
<tr>
<td>4</td>
<td>Presence of immune cells in the host</td>
<td>Present</td>
<td>Absent</td>
</tr>
<tr>
<td>5</td>
<td>Mode of action</td>
<td>The most through the channel formation in the cell cytoplasmic membrane</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Toxicity/other effects in eukaryotic cells</td>
<td>Absent</td>
<td>Present</td>
</tr>
</tbody>
</table>

Most of the bacteriocins of LAB characterized to date are small (< 10kDa) heat stable peptides, however, the occurrence of large (> 30kDa) heat labile proteins has also been reported (Joerger and Klaenhammer, 1986; Vaughan et al., 1992). Bacteriocins are extremely heat stable at low pH (Hurst, 1981; Hastings et al., 1991; Felix et al., 1994) becoming more sensitive to heat upon purification (Davey, 1981; Hastings et al., 1991). Bacteriocins of LAB, in general, are active over a wide pH range with optimum being on acidic side.

Currently, over 20 bacteriocins of lactic acid bacteria have been sequenced. Most contain less than 60 amino acids, and all are devoid of lipid and carbohydrate moieties. The molecules are cationic, hydrophobic and have isoelectric points ranging from 8.6 to 10.4. Their net positive charge varies with
pH, and this is important for both bactericidal efficiency and purification. (Ray et al., 2001). They also reported that due to the hydrophobic nature of these molecules they have a tendency to aggregate, especially when stored at high concentration or for a long time. Antibacterial potency is greater at lower pH, destroyed at a pH above 10, relatively heat stable but partially destroyed by heating above 100°C, and not affected after treatment with many organic and inorganic chemicals. However, some anions interfere with activity in a concentration dependent manner, and many proteolytic enzymes can hydrolyze the molecules resulting in loss of antibacterial properties.

Bactericidal potency remains stable during storage in dried or liquid forms at frozen and refrigeration temperatures. However, it slowly decreases during storage at room temperature in the presence of air that can oxidize methionine residues to the inactive methionine sulfoxide form (Ray 1992; Klaenhammer 1993; Jack et al., 1995; James et al., 1989; Jung et al., 1991b; Hoover and steenon, 1993; de-Vyust and Vandamme, 1994a; Yang et al., 1992; Ennahar et al., 2000b). Characteristics of some common bacteriocins produced from LAB are summarized in Table 4.

Table 4: Characteristics of some bacteriocins produced by LAB.

<table>
<thead>
<tr>
<th>Bacteriocin</th>
<th>Produced by</th>
<th>Number of amino acids</th>
<th>Molecular Weight (kDa)</th>
<th>Isoelectric points</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactococcin A</td>
<td><em>Lactococcus lactis</em> subsp. <em>cremonis</em></td>
<td>55</td>
<td>5.8</td>
<td>8.6</td>
<td>Van Beikum et al., (1991a)</td>
</tr>
<tr>
<td>Lactococcin B</td>
<td><em>Lactococcus lactis</em> subsp. <em>cremonis</em></td>
<td>47</td>
<td>5.3</td>
<td>9.1</td>
<td>Van Beikum et al., (1991a,b)</td>
</tr>
<tr>
<td>Sakacin P</td>
<td><em>Lactobacillus sake</em> LTH 673</td>
<td>43</td>
<td>4.4</td>
<td>8.8</td>
<td>Tichaczek et al., (1992)</td>
</tr>
<tr>
<td>Lactocin S</td>
<td><em>Lactobacillus sake</em> 245</td>
<td>37</td>
<td>3.9</td>
<td>N.A</td>
<td>Mortvedt and Nes, (1990)</td>
</tr>
<tr>
<td>Pediocin ACH</td>
<td><em>Pediococcus acidilactici</em> H</td>
<td>44</td>
<td>4.6</td>
<td>9.6</td>
<td>Bhunia et al., (1987b)</td>
</tr>
<tr>
<td>Leucocin A</td>
<td><em>Leuconostoc gelidium</em></td>
<td>37</td>
<td>3.9</td>
<td>9.5</td>
<td>Ahn and Stiles, (1990a,b, 1992)</td>
</tr>
</tbody>
</table>
The bacteriocins of lactic acid bacteria following translation usually undergo very little structural alteration, and thus, are regarded as ribosomally translated peptides. At the translation level, a molecule designated as prebacteriocin (such as prenisin and prepediocin) contains a leader peptide segment at the N-terminus and a propeptide segment at the C-terminus.

The molecules are transported out of the cytoplasm through the membrane by specific ABC (ATP binding cassette) transporters with the expense of energy. (Figure 6).

![Figure 6: Schematic depiction of transport of bacteriocins and signaling pathway leading to bacteriocin expression. IF, induction factor; HK, histidine kinase; P, phosphate; RR, response regulator; ABC, Trans, ABC transporter system; (Nes et al. 1996).](image)

During transport endopeptidase activity of the ABC transporter removes the leader peptide before the propeptide is released into the environment. (Jack et al., 1995; Liu and Hansen, 1990; Ennahar et al., 2000a). Ennahar et al., (2000b) reported that the fate of excised leader peptide is not known but it has been speculated that they might act as signaling molecules for the production of bacteriocins. It was assumed earlier that leader peptides, besides helping prebacteriocin molecules to interact with ABC transporters, also inhibit activity of the attached probacteriocin. Recent studies have shown that this may be true for nisin, but is not the case for pediocin PA-1/AcH, as prepediocin as well as pediocin PA1/AcH with a fused protein at the N-terminus are biologically active. (Ray et al., 1999; Miller et al., 1998; De-Vos et al., 1995a, b). Following release of the matured bacteriocin, it either remains
free or may bind via electrostatic attraction to the surface of the producer cells. At around pH 6, a large fraction of the molecules remain in the absorbed state while at pH 2 or below most are released into the environment (Yang et al., 1992).

The probactoicins part of some bacteriocins may undergo nonenzymatic as well as enzymatic chemical modifications. Pre-nisin molecules, while in the cytoplasm, undergo enzymatic dehydration of serine and threonine residues converting-

Serine to dhA (dehydroalanine, \( \text{CH}_2=\text{C} \quad \text{NH}_2 \quad \text{COOH} \)) and

Threonine to dhB (dehydrobutyrine, \( \text{CH}_3\cdot\text{CH}=\text{C} \quad \text{NH}_2 \quad \text{COOH} \))

Figure 7: The conversion of serine residues to threonine residues in cytoplasm

They then form thioether linkages to cysteine creating lanthionine (Lan; -Ala-S Ala) or β-methyl lanthionine (MeLan; - Abu-S-Ala). Bacteriocins with thioether rings, i.e., containing lanthionine and/or methyl lanthionine, are grouped as lantibiotics. In lantibiotics, these changes occur in the probactoicin sequence while the prebactoicin still resides in the cytoplasm. Lantibiotics can have different numbers of thioether rings e.g., nisin has five rings while lacticin 481 has three rings. The thioether rings play a crucial role in the antibacterial properties of a lantibiotic; however, the presence of lanthionine does not necessarily make a bacteriocin more potent than bacteriocin that lack lanthionine. (Ray et al., 2001).

2.4.4 Mode of action of bacteriocin: - Due to the great variety of their chemical structures, bacteriocins affect different essential functions of the living cell (transcription, translation, replication, and cell wall biosynthesis), but
most of them act by forming membrane channels or pores that destroy the energy potential of sensitive cells. The different modes of action of various types of bacteriocin produced by gram-positive bacteria have been reviewed by several authors (Sahl and Brandis, 1982; Abee, 1995; Ennahar et al., 2000).

"Nisin" (a compound belonging to group Ia, according to Klaenhammer's classification) is the bacteriocin whose mode of action has been studied the best. This cationic lantibiotic associates electrostatically with the negatively charged membrane phospholipids (Abee et al., 1995; Driessen et al., 1995), which favors subsequent interaction of bacteriocin's hydrophobic residues with the target cytoplasmic membrane. Lysine is the cationic amino acid involved in this electrostatic interaction, similar to the membrane interaction of mammal defensins mediated by arginine (Fujii et al., 1993). The interaction between the hydrophobic part of nisin and the bacterial target membrane generates unspecific ionic channels whose formation is aided by the presence of high transmembrane potentials, and by the presence of anionic and absence of cationic lipids (Hasncock, 1997). Pore formation, on the other hand, decreases in the presence of divalent cations (Mg2+ or Ca2+) because they neutralize the negative charges of the phospholipids, reducing the fluidity of the membrane. Nisin generated membrane pores allow the passive efflux of ions (K+ and Mg2+), amino acids (glutamic acid, lysin), and A TP, but not of larger cytoplasmic proteins, yielding membrane potential and proton-motiv-force dissipation and subsequent cell death (Boman et al., 1994).
**Figure 8: Mode of action of lactic acid bacteria bacteriocins.** Lactic acid bacteria (LAB) bacteriocins can be grouped on the basis of structure, but also on the basis of mode of action. Some members of the class I (or lantibiotic) bacteriocins, such as nisin, have been shown to have a dual mode of action. They can bind to lipid II, the main transporter of peptidoglycan subunits from the cytoplasm to the cell wall, and therefore prevent correct cell wall synthesis, leading to cell death. Furthermore, they can use lipid II as a docking molecule to initiate a process of membrane insertion and pore formation that leads to rapid cell death. A two-peptide lantibiotic, such as lactacin 3147, can have these dual activities distributed across two peptides, whereas mersacidin has only the lipid-II-binding activity, but does not form pores. In general, the class II peptides have an amphiphilic helical structure, which allows them to insert into the membrane of the target cell, leading to depolarisation and death. Large bacteriolytic proteins (here called bacteriolyssins, formerly class III bacteriocins), such as lysostaphin, can function directly on the cell wall of Gram-positive targets, leading to death and lysis of the target cell.

**Source:** - Cotter *et al.* (2005).
2.4.5 Antibacterial properties: - Many bacteriocin producing strains belonging to several genera and species of lactic acid bacteria have been isolated. Their bacteriocins are bactericidal to sensitive cells and death occurs very rapidly at a low concentration. Ray (1993) reported a range of Gram positive bacteria sensitive to a bacteriocin, while the producer strain is immune to its own bacteriocin and often is sensitive to other bacteriocins. Kalchyanand (1992) found that, although, Gram negative bacteria are normally resistant, they become sensitive to bacteriocins once their outer membrane is destabilized by physical, chemical or other stress.

Most of the class I bacteriocins have a fairly broad inhibitory spectrum. They not only inhibit closely related bacteria, such as species from the genera Enterococcus, Lactobacillus, Lactococcus, Leuconostoc, Pediococcus, and Streptococcus, but also inhibit many less closely related Gram-positive bacteria, such as Listeria monocytogenes, Staphylococcus aureus, Bacillus cereus, and Clostridium botulinum. Several bacteriocins in this class, such as nisin and thermophilin 13, prevent outgrowth of spores of Bacillus cereus and Clostridium botulinum. Interestingly, acidocin J1132 has a very narrow inhibitory spectrum and sensitive strains are limited to members of the genus Lactobacillus (Table 5), while at the other extreme, plantaricin LP84 (produced by Lactobacillus plantarum NCIM 2084) has demonstrated antagonism against E. coli (Suma et al., 1998).

Compared to class I bacteriocins, most class IIa bacteriocins have comparatively narrow activity spectra and only inhibit closely related Gram-positive bacteria. In general, members of the genera Enterococcus, Lactobacillus, Pediococcus are sensitive to class IIa bacteriocins, and members of the genus Lactococcus are resistant (Table 5). For example, Eijssink et al. (1998) found that pediocin PA-I was active against different species of Enterococcus, Lactobacillus, and Pediococcus; however, only 1 out of 11 Lactococcus strains tested (Lactococcus lactis LMG 2070) was sensitive to the bacteriocin. Some class IIa bacteriocins, such as pediocin PA-I, have fairly broad inhibitory spectra and can inhibit some less closely related Gram-
positive bacteria, such as *Staphylococcus aureus* and vegetative cells of *Clostridium* spp. and *Bacillus* spp. Some class Ila bacteriocins, such as mundticin from *Enterococcus mundtii*, even prevent the outgrowth of spores of *Clostridium botulinum* (Table 5).

As evident in Table 5, class Ila bacteriocins are generally active against *Listeria*. Eijsink et al. (1998) found that 9 strains of *Listeria* tested, including *Listeria monocytogenes*, *Listeria innocua* and *Listeria ivanovii* were very sensitive to 4 class Ila bacteriocins (pediocin PA-1, enterocin A, sakacin P, and curvacin A). Moreover, the extent of sensitivity varied from strain to strain. The minimal inhibitory concentrations against *Listeria monocytogenes* for the above 4 bacteriocins varied from 0.1 to 8 mg/ml, however, some *Listeria* strains, such as *Listeria monocytogenes* V7 and *Listeria innocua* LB 1, have been found to be resistant to class Ila bacteriocins (enterocin A, mesentericin Y I05, divercin V41, and pediocin AcH) (Ennahar et al., 2000a).

It might seem that bacteriocins with broader activity spectra would always be preferable for use in food preservation, but under certain circumstances bacteriocins with narrower inhibitory spectra may prove more desirable. For example, sakacin P, which has limited activity against LAB but nearly as effective as pediocin PA-1 against *Listeria*, might find application in LAB fermentation products that are prone to contamination by *Listeria monocytogenes* (Eijsink et al., 1998).
Table 5: Activity spectra of some Class I and Class IIA bacteriocins (Chen, H. and Hoover, D.G. (2003)).

<table>
<thead>
<tr>
<th>Bacteriocins</th>
<th>Strain</th>
<th>Activity spectra</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Class I</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acidocin J1132</td>
<td>Lactobacillus acidophilus JCM 1132</td>
<td>Active against different species of <em>Lactobacillus</em> (9/32)*, Not active against <em>Lactobacillus</em> (0/6), <em>Pediococcus</em> (0/5), <em>Streptococcus</em> (0/7), <em>Listeria monocytogenes</em> (0/4), <em>Bacillus</em> spp. (0/2), and <em>Staphylococcus</em> spp. (0/2).</td>
<td>Tahara et al., 1996</td>
</tr>
<tr>
<td>Plantaricin C</td>
<td>Lactobacillus plantarum LL441</td>
<td>Active against different species of <em>Enterococcus</em> (1/1), <em>Lactobacillus</em> (8/11), <em>Lactococcus</em> (1/1), <em>Leuconostoc</em> (2/12), <em>Pedococcus</em> (2/2), <em>Streptococcus</em> (2/12), <em>Staphylococcus carnosus</em> (1/1), <em>Bacillus</em> spp. (2/3), and <em>Clostridium</em> spp. (2/2). Not active against <em>L. innocua</em> (0/1)</td>
<td>Gonzalez et al 1994</td>
</tr>
<tr>
<td>Thermophilin 13</td>
<td>Streptococcus thermophilus SF113</td>
<td>Active against different species of <em>Enterococcus</em> (1/1), <em>Lactobacillus</em> (3/3), <em>Lactococcus</em> (1/1), <em>Leuconostoc</em> (2/2), <em>Streptococcus</em> (1/1), <em>L. monocytogenes</em> (1/1), <em>L. innocua</em> (1/1), <em>S. carnosus</em> (1/1), <em>Bacillus</em> spp. (2/2), and <em>Clostridium</em> spp. (2/2). Prevents outgrowth of spores of <em>B. cereus</em> and <em>Clostridium botulinum</em>.</td>
<td>Marciset et al 1997</td>
</tr>
<tr>
<td><strong>Class IIA</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acidocin A</td>
<td>Lactobacillus acidophilus TK9201</td>
<td>Active against different species of <em>Enterococcus</em> (1/5), <em>Lactobacillus</em> (13/32), <em>Pediococcus</em> (2/7), <em>Streptococcus</em> (8/13), and <em>L. monocytogenes</em> (5/5). Not active against <em>Bacillus subtilis</em> (0/6) and <em>S. aureus</em> (0/2).</td>
<td>Kanatani et al 1995</td>
</tr>
</tbody>
</table>

Table 5-Continued
<table>
<thead>
<tr>
<th>Agent</th>
<th>Species</th>
<th>Activity Notes</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bavarian A</td>
<td>Lactobacillus sake MI401</td>
<td>Active against different species of Enterococcus (2/2), Lactobacillus (11/25), Lactococcus (5/15), Leuconostoc (4n), Pediococcus (215), and L. monocytogenes (9/10). Not active against Carnobacterium (0/1), Streptococcus (0/2), Brochothrix thermosphacta (0/1), Bacillus spp. (On), and Staphylococcus spp. (0/5).</td>
<td>Larsen et al 1993</td>
</tr>
<tr>
<td>Curvacin A</td>
<td>Lactobacillus curvatus 1.THI1974</td>
<td>Active against different species of Carnobacterium (3/3), Enterococcus (1/2), Lactobacillus (10/23), Lactococcus (1/12), Pediococcus (5/8), L. monocytogenes (7n), L. innocua (1/1), and L. ivanovii (1/1). Not active against Leuconostoc (0/3) and Clostridium spp. (0/12).</td>
<td>Eijssink et al 1998</td>
</tr>
<tr>
<td>Divercin V41</td>
<td>Carnobacterium divergens V41</td>
<td>Active against different species of Enterococcus (4/4), Lactobacillus (2/5), Pediococcus (2/2), L. monocytogenes (1/1), L. innocua (1/1), and L. ivanovii (1/1). Not active against Lactococcus (0/1) and Leuconostoc (0/3).</td>
<td>Guyonnet et al 2000</td>
</tr>
<tr>
<td>Enterocin A</td>
<td>Enterococcus faecium CTC492</td>
<td>Active against different species of Enterococcus (4/4), Lactobacillus (2/2), Pediococcus (2/2), L. monocytogenes (4/4), and L. innocua (2/2).</td>
<td>Aymerich et al 1996</td>
</tr>
<tr>
<td>Lactococcin MMFII</td>
<td>Lactococcus lactis MMFII</td>
<td>Active against different species of Enterococcus (3/3), Lactobacillus (2/2), Lactococcus (2/6), and L. lanovii (1/1).</td>
<td>Ferchichi et al 2001</td>
</tr>
<tr>
<td>Mesentericin Y105</td>
<td>Leuconostoc mesenteroides Y105</td>
<td>Active against different species of Enterococcus (3/4), Lactobacillus (1/5), Leuconostoc (2/3), Enterococcos (2/2), L. monocytogenes (1 1), L. innocua (1/1), and L. ivanovii (1/1). Not active against Lactococcus (0/1)</td>
<td>Guyonnet et al 2000</td>
</tr>
<tr>
<td>Mundtacin</td>
<td>Enterococcus mundtii ATO6</td>
<td>Active against different species of Carnobacterium (1/1), Enterococcus (2/2), Lactobacillus (2/2), Leuconostoc (2/2), Pediococcus (2/2), L. monocytogenes (1/1), and L. innocua (1/1). Prevents the outgrowth of spores and vegetative cells of C. botulinum.</td>
<td>Bennik et al 1998</td>
</tr>
<tr>
<td>Pediocin PA-1</td>
<td>Pediococcus acidilactici PAC 1.0</td>
<td>Active against different species of Carnobacterium (3/3), Enterococcus (2/3), Lactobacillus (23/31), Lactococcus (1/14), Leuconostoc (3/4), Pediococcus (8/11), L. monocytogenes (12/12), L. innocua (2/2), L. ivanovii (1/1), Staphylococcus spp. (2/6), B. cereus (1/1), and Clostridium spp. (4/17).</td>
<td>Cintas et al 1998; Eijssink et al 1998</td>
</tr>
<tr>
<td>Piscicin V1a</td>
<td>Carnobacterium piscicola V1</td>
<td>Active against different species of Carnobacterium (2/2), Enterococcus (1/1), Lactobacillus (3/3), Leuconostoc (1/1), Pediococcus (1/1), L. monocytogenes (1/1), and L. innocua (1/1). Not active against Lactococcus (0/1), B. cereus (0/1), Clostridium spp. (0/3), and S. aureus (0/1).</td>
<td>Bhugaloo-Vial et al 1996</td>
</tr>
<tr>
<td>Piscicin V1b</td>
<td>Carnobacterium piscicola V1</td>
<td>Active against different species of Carnobacterium (2/2), Enterococcus (1/1), Lactobacillus (3/3), Leuconostoc (1/1), Pediococcus (1/1), L. monocytogenes (1/1), and L. innocua (1/1). Not active against Lactococcus (0/1), B. cereus (0/1), Clostridium spp. (0/3), and S. aureus (0/1).</td>
<td>Bhugaloo-Vial et al 1996</td>
</tr>
</tbody>
</table>
### Table 5-Continued

<table>
<thead>
<tr>
<th>Piscicolin 126</th>
<th>Carnobacterium piscicola JG126</th>
<th>Active against different species of Carnobacterium (1/1), Enterococcus (2/2), Lactobacillus (2/3), Leuconostoc (2/3), Pediococcus (1/2), Streptococcus (2/2), L. monocytogenes (2/2), L. grayi (1/1), L. ivanovii (1/1), L. seeligeri (1/1), and B. thermosphacta (1/1). Not active against Bacillus spp. (0/5), Clostridium spp. (0/2), Lactococcus (0/3), Listeria denitrificans (0/1), and Staphylococcus spp. (0/3).</th>
<th>Jack et al 1996</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sakacin A</td>
<td>Lactobacillus sake LB706</td>
<td>Active against different species of Enterococcus (7/8), Lactobacillus (3/17), Pediococcus (1/4), L. monocytogenes (5/5), L. innocua (3/3), and L. ivanovii (1/1). Not active against Lactococcus (0/1) and Leuconostoc (0/3).</td>
<td>Aymeric et al 1996; Guyonnet et al 2000</td>
</tr>
<tr>
<td>Sakacin P</td>
<td>Lactobacillus sake LB674</td>
<td>Active against different species of Enterococcus (7/8), Lactobacillus (3/17), Pediococcus (2/4), L. monocytogenes (5/5), L. innocua (3/3), and L. ivanovii (1/1). Not active against Lactococcus (0/1) and Leuconostoc (0/3).</td>
<td>Aymeric et al 1996; Guyonnet et al 2000</td>
</tr>
</tbody>
</table>

*the number inside the bracket represent “Number of strains inhibited/Numbers of strains tested

Several other characteristics of bacteriocins and bacteriocin producing lactic acid bacteria have been noted (Ray 1992; Ennrah 2000b; Ray and Miller, 2000). A strain can sometimes produce more than one type of bacteriocin (e.g. lactocin A, B and M by Lactococcus lactis subsp. cremoris). Strains of the same species generally produce the same bacteriocin (e.g. pediocin PA-1/AcH by different Pediococcus acidilactici strains), however, strains of the same species can also produce different bacteriocins (e.g. Sakacin A and Sakacin P produced by two strains of Lactobacillus sake), and the strains from different species and different genera can produce the same bacteriocin (e.g., pediocin PA-1/AcH produced by Pediococcus acidilactici, Pediococcus pentosaceus, Pediococcus parvulus, and Lactobacillus plantarum strains). Strains from different subspecies of the same species can produce different bacteriocins (e.g. nisin A and lactocin 481 produced by different strains of Lactococcus lactis subsp lactis) different species in a genus can produce different bacteriocins (e.g. Enterococcin EFS2 and enterocin 900 produced by strains of Enterococcus faecalis and Enterococcus faecium, respectively). Natural variants of the same bacteriocin can be produced by different strains of the same species (e.g. nisin A and nisin Z by Lactococcus...
lactis subsp. lactis, strains ATCC 11454 and ATCC 7962, respectively) and also by different species (e.g. leucocin A and mesenterocin by *Leuconostoc gelidum* and *Leuconostoc mesenteroides*, respectively). These generalizations are drawn based on analysis of the amino acid sequences of numerous bacteriocins.

### 2.4.6 Production of bacteriocins:

One of the most important steps in the study of bacteriocins is their production. The composition of culture medium and cultural conditions such as temperature, pH and time of incubation have profound effect on the production of bacteriocins. In general, conditions that provide high cell density favour high bacteriocin concentration.

The culture media generally employed for the growth of lactic acid bacteria such as MRS, APT, TGE, M17G, ELB etc. have also been found to support good bacteriocin production. Although, bacteriocin production occurs over a wide temperature range, it is greater at the optimum temperature for the growth of the producer.

The production of bacteriocins by lactic acid bacteria is strongly influenced by the pH of the culture medium. The regulation of pH at a certain value during the course of fermentation has been found to have favourable (Hurst, 1981; Piard et al., 1990) and detrimental (Biswa et al., 1991; Coventry et al., 1996) effects on the final yield of bacteriocins of lactic acid bacteria.

The maximum production of bacteriocins occurs at different phases in the cell growth cycle. Most of the bacteriocins of lactic acid bacteria are secreted during the logarithmic growth phase with a slight decline in the activity of some of them during the stationary phase of the producer culture. However, some bacteriocins for e.g. nisin (Hurst, 1981), pediocin SJ-1 (Schved et al., 1993) are secreted as secondary metabolites. The termination of the incubation at appropriate time is essential to prevent the loss of bacteriocin activity.
2.4.6.1 Growth medium: - Commonly used media for the production of bacteriocins by lactic acid bacteria include MRS (ten Brink et al., 1994; Coventry et al., 1996; Holo et al., 2001; Vaughan et al., 2001; Luca et al., 2002; Pu et al., 2002; Leal-Sanchez et al., 2002; Yukio et al., 2003; Antonio et al., 2003; Savadogo et al., 2004; Oyetayo, 2004; Yanagida et al., 2005; Fugen et al., 2005; Kang and Lee, 2005; Hernandez et al., 2005; Cock and Stoutenel, 2006), TGE (Biswa et al., 1991; Yang and Ray, 1994b), APT (Lewus et al., 1992), GM17 (Parente and Hill, 1992; Izildinhia et al., 1999; Beatriz et al., 2000; Izildinhia et al., 2000), M17 (Stiles and Hastings, 1991; Izildinhia et al., 2000; Pu et al., 2002; Guessas and Kihal, 2004; Rossetti and Giraffa 2005; Achemchem et al., 2005), ELB (Geis et al., 1983; Piard et al., 1990), BHI (Bizani et al., 2005; Achemchem et al., 2005) etc. with or without modifications. Although, a large number of bacteriocins have been found to be identified and several media have been used for the production of bacteriocins, very few studies are available on the comparision of bacteriocin production in different media.

Geis et al. (1983) compared various media including ELB, GM17, BHI, a synthetic medium and milk for their ability to support bacteriocin production by various lactococcal strains. All the strains produced antibiotic activities in milk. Highest bacteriocin activities were found in unbuffered ELB followed by BHI, buffered M17 and synthetic medium (Geis et al., 1983).

Lactococcus lactis subsp. lactis CNRZ481 produced maximum bacteriocin (12800 AU/ml) in ELB buffered with sodium β-glycerophosphate. The observed titre was double than the value recorded when the culture was grown in M17 or unbuffered ELB (Piard et al., 1990).

Parente and Hill (1992) formulated three media (Tryptone-Yeast Extract-Tween) TYT10, TYT11 and TYT30 and compared with seven different media [ELB, M17, M17 dialysate, Tryptose phosphate (TP), tryptone yeast extract broth (TYB), yeast glucose lecme (YGL) broth and MRS] for the growth of and bacteriocin production by Lactococcus lactis subsp. lactis DPC3286 and Lactococcus lactis subsp. cremoris LMG2130. Good growth and
bacteriocin production were obtained for both in the TYT, M17 and MRS media. Bacteriocin production was very poor in YGL. It was also observed that Lactococcus lactis subsp. cremoris LMG2130 could not grow or produce bacteriocins in M17 dialysate and TP media (Parente and Hill, 1992). Although the cell mass was greater in MRS broth, 15% less pediocin ACh production by P. acidilactici LB42-923 produced higher pediocin ACh titres in TGE broth than in buffered TGE broth (Yang and Ray, 1994b).

In contrast to pediocin ACh, higher levels of nisin, sakacin A and leuconocin Lm1 were observed in TGE buffer broth than in TGE (Yang and Ray, 1994b). Earlier Hechard et al. (1992) observed consistently higher levels of (x16) mesentericin Y105 in MRS broth than in a semi-defined medium.

2.4.6.2 Effect of pH on bacteriocins production: -

2.4.6.2.1 Lactobacilli bacteriocins:-- Barefoot and Klaenhammer (1984) reported maximum lacticin B production when Lactobacillus acidophilus N2 was grown in MRS broth regulated at pH 6.0. In contrast, lacticin F was produced maximally in MRS broth held at a constant pH of 7.0 rather than 7.5, 6.0 or 5.0 (Muriana and Klaenhammer, 1987). Production of heveticin J and helveticin V-1829 was observed to be greatest in anaerobic MRS cultures maintained at a pH 5.5 than at other pH values tested in the range of 5.0 to 7.0 (Joerger and Klaenhammer, 1986; Vaughan et al., 1992). Vaughan et al. (1992) also reported a two-fold increase in helveticin V-1829 when MRS broth was held at a pH 5.0 than in pH-unregulated cultures.

Ten-Brink et al. (1994) observed that growth of Lactobacillus acidophilus M46 in five fold concentrated MRS broth held at a constant pH 5.5 resulted in eight fold increase in acidocin B activity than that obtained after growth in normal MRS broth without pH control. Regulation of MRS broth at pH 5.0 resulted in maximum yield of acidocin A produced by Lactobacillus acidophilus TK9201 (Kanatani et al., 1995).

Maximum production of plantaricin S was obtained in a fermenter system in unregulated pH in MRS broth containing 4% NaCl. It was also
reported that regulation of pH at 4.0-7.0 during fermentation had a detrimental effect on the production of plantaricin S by *Lactobacillus plantarum* LPC010 (Jimenez-Diaz et al., 1993).

Coventry et al. (1996) studied the effect of pH on the production of brevicin 286 by *Lactobacillus brevis* VB286. No substantial cell growth or brevicin 286 activity was detected in MRS broth with an initial pH 4.5. In spite of substantial cell growth, brevicin 286 production was minimal at pH 5.0. Optimum production of brevicin 286 was observed in MRS broth at an initial pH of 6.0-6.5. It was also observed that regulation of pH at either 6.0 or 6.5 had no advantage over stirred culture without pH control with respect to brevicin 286 (Coventry et al., 1996).

### 2.4.6.2.2 Lactococcal bacteriocins:

Nisin production was maximum when medium was maintained at pH 6.0 alongwith a large cell mass (Hurst, 1981). Piard et al. (1990) observed maximum lacticin 481 production when the producer strain *Lactococcus lactis* subsp. *lactis* CNRZ481 was grown in buffered ELB 6.5 or growing the producer in pH non-regulated medium resulted in decreased bacteriocin yields (Piard et al., 1990). Bacteriocin production by *Lactococcus lactis* subsp. *lactis* ADRI 85L030 was reported to be independent of the initial pH of the medium in the range 5.0 to 7.0 (Thuault et al., 1991). Cock and Stouvenel in 2006 reported MRS liquid culture medium, after 48 h at 36°C and 45°C in anaerobic condition for lactic acid production by a strain of *Lactococcus lactis* subsp. *lactis* isolated from sugarcane plants.

### 2.4.6.2.3 Leuconostocs bacteriocins:

*Leuconostocs gelidum* UAL187 produced leucocin A-UAl187 maximally in APT broth at pH 6.0 and 6.5. At a lower initial pH, growth of the producer organism was slower and a concentration maximum was lower (Hastings and Stiles, 1991). Lewus et al. (1992) studied the effect of initial pH of APT broth on the growth of and bacteriocin production by *Leuconostoc paramesenteroides* OX. Leuconocin
Swas produced in detectable amounts at pH 6.0 and appeared to be optimal (400 AU/ml) at pH 6.5 and 7.0. They observed slight depression in growth and leuconocin S production at pH 7.5. Recently Baker et al. (1996) reported that the production of leuconocin S was maximum (2000 AU/ml) in fermenters maintained at pH 7.0 than at 6.0, 6.5 and 7.5. Van Laack et al. (1992) observed a 50% decrease in the production of carnosin 44A when the initial pH of MRS broth was lowered from 6.0 to 5.1.

Although, Leuconostoc carnosum Talla produced leucin B-Talla in MRS broth with an initial pH in the range 4.5 to 7.5, the bacteriocin concentration was found to be optimal at pH 6.0 to 6.5 (Felix et al., 1994).

2.4.6.2.4 Pediococcal bacteriocins:- Pediococcus acidilactici H produced maximum pediocin AcH when grown in TGE broth with an initial pH of 6.5. Pediocin AcH was produced in negligible amounts when the pH of TGE broth was maintained at pH 5.0 or above. It was concluded that a terminal pH below 4.0 along with a large cell mass was essential for the production of pediocin AcH (Biswaas et al., 1991). High titres of pediocin N5P were observed when P. pentosaceus N5P was grown in TGE broth with an initial pH of 6.5 (Strasser-de-saad and Manca-de-Nadra, 1993). It was also reported that pediocin N5P could not be detected in TGE broth at an initial pH below 5.0. Liao et al. (1993) reported optimum production of pediocin PO2 in whey permeate medium with an initial pH of 6.5 without pH regulation during incubation.

2.4.6.3 Effect of temperature on bacteriocins production:-
2.4.6.3.1 Lactococcal bacteriocins:- Nisin production was maximum when the culture was incubated between 25 and 30°C as opposed to 37°C. Incubation of nisin producer at 37°C resulted in 386 AU/ml of nisin as compared to 542 AU/ml at 26°C (Hurst, 1981). Thuault et al. (1991) reported that the bacteriocin production by Lactococcus lactis subsp. lactis ADRI 85L030 was not significantly dependent on the incubation temperature in the range of 30 to 42°C.
2.4.6.3.2 *Leuconostocs* bacteriocins:- *Leuconostoc camosum* Talla produced bacteriocin, leucocin B-Talla over a wide range of temperature i.e. 0°C to 30°C, but the optimal production was observed at 25°C (Felix et al., 1994). Van Laack et al. (1992) reported that *Leuconostoc camosum* LA44A could grow and produce bacteriocins in the temperature range of 4-10°C. Although bacteriocins by various *Leuconostoc* spp. was observed both at 4°C and 25°C, the bacteriocin titres, in general, were 2-3 times higher at 25°C than at 4°C (Yang and Ray, 1994a). Leucocin A-UAL187 production by *Leuconostoc gelidum* UAL187 was observed over a wide range of incubation temperatures (1-25°C) with more time taken at low temperatures (Hastings and Stiles, 1991).

2.4.6.3.3 Pediococcal bacteriocins:- *Pediococcus acidilactici* H produced same amounts of pediocin AcH after 16 h of growth in TGE broth both at 30°C and 37°C. The cell mass and bacteriocin production were slightly reduced at 40°C (Biswas et al., 1991). Schved et al. (1993) observed the production of pediocin SJ-1 at 20°C, 30°C, 40°C and 45°C with optimal production in the range of 35 to 40°C. It was reported that the maunt of pediocin L50 fromed at 16°C was comparable to that formed at 32°C, while considerably less amount was produced at 8°C. The organism failed to produce detectable amounts of bacteriocin at 45°C (Cintas et al., 1995).

2.4.6.4 Growth phase:-

2.4.6.4.1 Lactobacilli bacteriocins:- Joeger and Klaenhammer, (1986) observed accumulation of helveticin J between late log phase and stationary phase of growth of *Lactobacillus helveticus* 481. Helveticin V-1829 was produced from the middle log phase into the stationary phase of growth of *Lactobacillus helveticus* V-1829 (Vaughan et al., 1992).
Barefoot and Klaenhammer (1984) observed the production of lacticin B during the logarithmic phase of growth of *Lactobacillus acidophilus* N2. *Lactobacillus acidophilus* M46 produced acidocin B continuously during the logarithmic growth phase. The level of inhibition reached maximum at the beginning of the stationary phase and maintained constant for at least 24 h (ten Brink et al., 1994).

*Lactobacillus plantarum* C-11 was found to accumulate maximum amount of plantaricin A during the mid log phase of growth with a decrease in activity thereafter (Daeschel et al., 1990). Maximum production of plantaricin S was obtained in log phase cultures of *Lactobacillus plantarum* LPC010. It was also observed that *Lactobacillus plantarum* PLC010 secreted another bacteriocin-designated plantaricin T in the late-stationary phase (Jimenez-Diaz et al., 1993). Rekhif et al. (1994) reported plantaricin LC74 production in exponential phase of growth of *Lactobacillus plantarum*, however, the bacteriocin, plantaricin KW30, was maximally produced at the beginning of stationary phase culture of *Lactobacillus plantarum* KW30 (Kelly et al., 1996).

It was reported that the concentration of brevicin 286 was highest at the late exponential growth phase (Coventry et al., 1996).

**2.4.6.4.2 Lactococcal bacteriocins:** Davey and Pearce (1980) observed diplococcin production by *Lactococcus lactis* subsp. *cremonis* 346 throughout the exponential growth phase. Nisin is synthesized as a secondary metabolite at a high rate when the cells have reached mid-exponential phase, and continues to be synthesized during a greater part of the stationary phase when the cells are grown at a constant pH of 6.8 at 30°C for 20 to 24 h (Ray, 1992a).

Bacteriocin S50 by *Lactococcus lactis* subsp. *lactis* biovar. *diacetyltactis* S50 was produced continuously during the growth, but the highest production was observed after 8 h of incubation (Kojic et al., 1991). Lacticin 481 production occurred in late-log phase of growth of *Lactococcus lactis* subsp. *lactis* 481 (Piard et al., 1990).
2.4.6.4.3 *Leuconostoc* bacteriocins:-- Mathieu *et al.* (1993) reported that the biosynthesis of mesenterocin 52 and its secretion into the medium started early in the growth phase, continued over the whole of that phase before reaching a maximum at the end. A decrease up to one to two orders of magnitude in the activity of carnocin LA54A was recorded during the stationary phase (Keppler *et al.*, 1994). Yang and Ray (1994a) observed the termination of bacteriocins production by various *Leuconostoc* spp. in the stationary phase of their growth. Leucocin B-Talla production occurred during the exponential phase of growth of the producer *Leuconostoc carnosum* Talla (Felix *et al.*, 1994). Production of leucocin A-UAL187 occurred early in the growth cycle of the producer organism, rather than as secondary metabolites of growth (Hastings and Stiles, 1991).

2.4.6.4.4 *Pediococcal* bacteriocins:-- Biswas *et al.* (1991) reported that about 60% of the pediocin AcH was produced by 8 h and the rest 40% was produced during the next 8 h (stationary phase). The authors have suggested that pediocin AcH appeared to be a secondary metabolites. Later studies have shown that post translational processing of prepediocin to active pediocin AcH occurred efficiently at a pH below 5.0 (Johnson *et al.*, 1992). After 24 h of growth, the pediocin AcH was slightly reduced at all the temperatures studied (Biswas *et al.*, 1991). Production of pediocin during the logarithmic and early stationary phases of growth suggested that pediocin SJ-1 was a secondary metabolite and after reaching maximum levels, in contrast to many bacteriocins, the antibacterial activity of pediocin SJ-1 remained stable in broth cultures over a period of up to 48 h (Schved *et al.*, 1993). *Pediococcus acidilactici* L50 produced highest bacteriocin from the onset of stationary phase and it remained stable at 8°C and 16°C while at 32°C, a decrease in antibacterial activity was seen throughout the stationary phase (Cintas *et al.*, 1995). Daba *et al.* (1991) observed the secretion of pediocin 5 from *P. acidilactici* UL5 during the late exponential phase of growth and the activity dropped sharply (>90% in 24 h) during the early stationary phase, however,
experiments with pH controlled at 5.0 did not show this large decrease in activity during the stationary phase.

2.4.7 Purification of bacteriocins: - An extensive characterization with respect to physical and chemical properties of bacteriocins is necessary before considering them for application in foods. The availability of bacteriocins in a pure form is essential for characterization studies.

Purification of bacteriocins is a difficult task for several reasons. Firstly, protein concentration in the supernatant is very high while bacteriocin concentration is low, meaning a very low specific activity. Secondly, bacteriocins form a heterogeneous group of substances, and the specific purification protocol has to be developed by trial and error for each bacteriocin. An additional problem encountered with the purification of bacteriocins of lactic acid bacteria is the use of media containing tween 80, a surfactant that has been shown to interfere with the precipitation procedures (Murriana and Klaenhammer, 1991a; van Laack et al., 1992). Vaughan et al. (2001) purified the bacteriocin to homogeneity by ammonium sulphate precipitation, cation exchange, hydrophobic interaction and reverse-phase liquid chromatography.

During the recent years, the above mentioned problems have been overcome and several bacteriocins of lactic acid bacteria have been purified to homogeneity by growing the producers in semi-defined media by minimizing the level of contaminating proteins and peptides (Joerger and Klaenhammer, 1986; Hastings et al., 1991; Hechard et al., 1992). Also MRS broth has been generally modified by omission of tween 80 (van Laack et al., 1992; Mortvedt et al., 1991).

2.4.7.1 Lactobacilli bacteriocins: - Barefoot and Klaenhammer, (1984) purified lacticin B by ion-exchange chromatography, ultrafiltration and gel filtration chromatography. Later, as mentioned by Nettles and Barefoot, (1993) a simpler purification protocol was devised for lacticin B. The protocol involved lyophilisation of culture supernatants followed by ultrafiltration and preparative
electrofocussing. Muriana and Klaenhammer, (1991a) achieved a 474-fold increase in specific activity of lactacin F by ammonium sulfate precipitation, gel filtration and HPLC.

Lactocin S produced by Lactobacillus sake L45 was purified to a 4000-fold increase in specific activity with a recovery of just 3.0% by ammonium sulfate precipitation, and sequential anion and cation exchange, hydrophobic interaction, gel filtration, phenyl superose and reverse-phase chromatographies (Mortvedt et al., 1991). Holck et al. (1992) purified sakacin A to a 9000-fold increase in specific activity and a very good recovery of about 80% was achieved by ammonium sulfate precipitation, ion exchange, hydrophobic interaction and reverse-phase chromatography.

Plantaricin S from plantarum LPC010 was purified to homogeneity by ammonium sulfate precipitation, binding to SP-sepharose fast flow, phenyl sepharose CL-4B and C2/C-18 reverse-phase chromatographies. The purification protocol resulted in a final yield of 91.6% and 352, 617-fold increase in specific activity (Jimenez-Diaz et al., 1995).

A purification protocol comprising ammonium sulfate precipitation and sequential cation exchange and reverse-phase chromatographies has been used for the purification of acidocin A with a recovery of about 10% (Kanatani et al., 1995). The protocol resulted in a more than 3000-fold increase in the specific activity of acidocin A.

2.4.7.2 Lactococcal bacteriocins:- Diplococcin was purified from the supernatant of Lactococcus lactis subsp. cremoris 346. The procedure employed included ammonium sulfate precipitation (60% saturation) and cation exchange chromatography on carboxy methyl cellulose (CMC) resulting approximately 1000-fold purification (Davey and Richardson, 1981). Dufour et al. (1991) purified lactococcin from culture supernatant of Lactococcus lactis subsp. lactis as a single band by dialysis, cation exchange and gel filtration chromatographies. The procedure employed resulted in a 14.5-fold purification with about 3000-fold increase in specific activity.
Ammonium sulfate precipitation of culture supernatant obtained from *Lactococcus lactis* subsp. *lactis* CNRZ481 resulted in a 455-fold increase in the total lacticin 481 activity. Subsequent purification by gel filtration chromatography and C18 reverse-phase high performance liquid chromatography (HPLC) lead to a 107,506-fold increase in the specific activity of lacticin 481 (Piard et al., 1992). Holo et al. (1991) purified lacticoccin A with about 2300-fold purification and yield of 16% by a sequential protocol including ammonium sulfate precipitation, cation exchange chromatography and reverse-phase HPLC. Lacticoccin G was similarly purified to homogeneity by a four step protocol which included ammonium sulfate precipitation, binding to a cation exchanger and octyl-sepharose CL-4B and reversed-phase chromatography leading to a recovery of about 20% of the original activity and a 7000-fold increase in specific activity (Nissen-Meyer et al., 1992). The bacteriocin diacetin B produced by *Lactococcus lactis* subsp. *lactis* biovar.diacetylactis UL720 was purified by a pH dependent adsorption-desorption procedure followed by a reverse-phase HPLC with a yield of just 1.25% of the original activity (Ali et al., 1995).

2.4.7.3 *Leuconostocs* bacteriocins- Leucocin A-UAL187 from *Leuconostoc gelidium* UAL-187 was purified by ammonium sulfate precipitation followed by a sequential hydrophobic interaction, gel filtration and reverse-phase HPLC with a yield of 58% of the original activity and a purification fold of 4500 (Hastings et al., 1991). Hechard et al. (1992) employed a three-step protocol for the purification of mesentericin Y105. The protocol included affinity chromatography on a blue agarose column, untrafiltration through a 5-kDa cut off membrane and finally reverse-phase HPLC on a C4 column. The purification procedure resulted in a very low yield of 0.7% with a purification fold of about 420. The purification procedure consisting of ammonium sulfate precipitation, and a sequential gel filtration, cation exchange and hydrophobic interaction chromatography resulted in a satisfactory increase of specific activity (1,135-fold) but a very low recovery of 8% of mesenterocin 52.
produced by *Leuconostoc mesenteroides* FR52 (Sudirman *et al.*, 1994). Keppler *et al.* (1994) reported the purification to homogeneity of carnocin LA54A by single step hydrophobic interaction chromatography using amberlite XAD-2. Revol-Juneless and Lefebvre, (1996) reported the purification of dextrancin J24 to homogeneity by desorbing the bacteriocin from the producer cells at pH 2.0 followed by a reverse-phase HPLC.

### 2.4.7.4 Pediococcal bacteriocins: -

Pediocin AcH from the culture supernatant of *P. acidilactici* H was purified by ammonium sulfate precipitation (70% saturation), fast protein liquid chromatography (FPLC), gel filtration and anion exchange chromatography leading to a 98.8-fold purification with a single band on SDS-PAGE gel (Bhunia *et al.*, 1988). Yang *et al.* (1992) reported the purification of pediocin AcH to homogeneity as revealed by a single sharp band on SDS-PAGE gel by a pH dependent adsorption/desorption procedure. Pediocin AcH was adsorbed to the producer cells at a pH of 6.0-6.5, centrifuged; the bacteriocin adsorbed onto the cells was extracted at a low pH of 1.5-2.0. The purification protocol resulted in the recovery of almost all the bacteriocin produced. Henderson *et al.* (1992) reported a 470-fold purification of pediocin PA-1 by gel filtration, ion-exchange chromatography, dialysis and HPLC, whereas Lozano *et al.* (1992) achieved a 80,000-fold increase in specific activity of pediocin PA-1 by employing ammonium sulfate precipitation, chromatography with a cation exchanger and octyl sepharose and reverse-phase HPLC.

Daba *et al.* (1994) employed the pH dependent adsorption/desorption procedure developed by Yang *et al.* (1992) for the recovery of pediocin 5 produced by *P. acidilactici* UL5. The procedure resulted in a partial recovery of the cell associated bacteriocin fraction and even longer desorption times exceeding 24 h could not result in the recovery of more than 10% of the original activity. Further purification to homogeneity was, however, achieved by reverse-phase HPLC (Daba *et al.*, 1994).
Schved et al. (1993) reported a 262-fold purification with a recovery of 50% of pediocin SJ-1 by the direct application of cell free supernatant containing crude bacteriocin to a cation exchange chromatography column. The homogeneity of pediocin SJ-1 thus purified was confirmed by SDS-PAGE. Cintas et al. (1995) purified pediocin L50 to homogeneity by ammonium sulfate precipitation, and sequential cation exchange, hydrophobic interaction and reverse-phase chromatographies resulting in the recovery of more than 80% of the starting material with a 114, 112-fold increase in specific activity.

2.4.8 Genetic determinants of bacteriocin production and immunity: - An understanding of the genetic control for bacteriocin production and host immunity might be beneficial for their effective use. This is also necessary for cloning and sequencing of genes involved, and application of genetic methods for the construction and improvement of bacteriocin producing strains of LAB.

The original criteria laid down for bacteriocins specify the plasmid borne genetic determinants of bacteriocin production and host cell immunity (Tagg et al., 1976). Although, most of the bacteriocins of lactic acid bacteria analysed to date adhere to this criterion, a very few, especially those produced by lactobacilli, have been found to have chromosomal borne genetic determinants (Barefoot and Klaenhammer, 1983; Joerger and Klaenhammer, 1986; Thompson et al., 1996). The bacteriocin immunity genes are generally borne on the plasmids that encode bacteriocin production, however, bacteriocin plasmids that do not carry immunity genes have also been found in lactic acid bacteria (Gonzalez and Kunka, 1987; Schved et al., 1993; Kanatani and Oshima, 1994).

2.4.9 Chemical nature of bacteriocins: - All bacteriocins, which have been studied in sufficient detail, are found to be macromolecular particulate in nature and include, if not consist of polypeptides or protein. Currently, over 20 bacteriocins of lactic acid bacteria have been sequenced. Most contain less than 60 amino acids and all are devoid of lipid and carbohydrate materials.
The molecules are cationic and hydrophobic. Due to the hydrophobic nature of these molecules they have a tendency to aggregate, especially when stored at high concentration or for a long time (Ray and Miller, 2001).

Their high isoelectric point (Jack et al., 1995) allows them to interact at physiological pH values with the anionic surface of bacterial membranes. This interaction can suffice, in the case of broad-spectrum bacteriocins, or facilitate, in the case of receptor-requiring compounds, insertion of the hydrophobic moiety into the bacterial membrane. Later, the cooperation between a number of bacteriocin molecules will build up the transmembrane pore responsible for gradient dissipation and cellular death. These features have favored the development of general purification protocols for bacteriocins that include hydrophobic interaction, cationic exchange and reverse-phase chromatographic steps (Shafer, 1997). The complex pattern of monosulfide and disulfide intramolecular bonds helps in the stabilization of secondary structures by reducing the number of possible unfolded structures (entropic effect). From a structural point of view, the effect of the intramolecular bonds is additive, and the higher their number, the higher the global stability of the peptide (Branden and Tooze, 1991). These facts and the sensitivity of nisin to digestive enzymes discouraged the clinical application of this compound but made it a product of choice as food preservative (Barnby-smith, 1992).

The studies on the kinetics of killing by bacteriocins show that killing begins as soon as the bacteriocin is added to a culture and suggest that all bacteriocins are bacteriocidal, as opposed to bacteriostatic (Reeves, 1965). Bacteriocidal effect remains stable during storage in dried or liquid forms at frozen and refrigeration temperatures. It slowly decreases during storage at room temperature in the presence of air that can oxide methionine residues to the inactive methionine sulfoxide form Molecules with disulphide bond can undergo bond exchange in the presence limited amounts of reducing agent and form dimer and trimers that retain bactericidal activity. (Ray, 1992; Klaenhammer, 1993; Hansen, 1990; Ennahar, 2000b). The first stage is specific irreversible adsorption of the bacteriocion and that in some instances
only one molecule may be required to kill a sensitive cell (Jacob et al., 1953; Nomura, 1963). Bactericidal nature of bacteriocins has also been observed by Toora et al. (1994), as inhibition zone remained clear of indicator colonies for two weeks in case of Yersinia enterocolitica.

2.4.10 Bacteriocin biosynthesis: - Bacteriocins are synthesized as pre-propeptide which are processed and externalised by dedicated transport machinery (Nes et al., 1996). Bacteriocin production in LAB is growth associated: it usually occurs throughout the growth phase and ceases at the end of the exponential phase or sometimes before the end of growth (Parente et al., 1997; Lejeune et al., 1998). Bacteriocin production is affected by type and level of the carbon, nitrogen and phosphate sources, cations surfactants and inhibitors. Bacteriocins can be produced from media containing different carbohydrate sources. Nisin Z can be produced from glucose, sucrose and xylose by Lactococcus lactis IO-1 (Matsuaki et al., 1996; Chinachoti et al., 1997a, b) but better results were obtained with glucose compared to xylose. Glucose followed by sucrose, xylose and galactose were the best carbon sources for the production of Pediocin AcH in an unbuffered medium Biswas et al., 1991).

All bacteriocins are synthesized with an N terminal leader sequence and until recently only the double glycine type of leader was found in class II bacteriocins (Holo et al., 1991; Muriana and Klaenhammer, 1991; Klaenhammer, 1993; Havarstein et al., 1994). However, it has now been disclosed that some small, heat stable and non modified bacteriocins are translated with sec dependent leaders (Leer et al., 1995; Worobo et al., 1995). The structural bacteriocin gene encodes a preform of the bacteriocin containing an N-terminal leader sequence (termed double glycine leader) whose function seems to prevent the bacteriocin from being biologically active while still inside the producer and provide the recognition signal for the transporter system.
A number of genes, often found in close proximity to each other are required for production of lantibiotics. These genes include:

(a) The structural gene, lan A,
(b) Immunity genes (Lan I and in some cases Lan E, Lan F and Lan G) encoding proteins that protect the producer from the producer lantibiotic,
(c) A gene Lan T encoding what appears to be a membrane associated ABC transporter that transfers, the lantibiotic across the membrane,
(d) A gene, lan P, encoding a serine proteinase, which removes the leader sequence of the lantibiotic prepeptide,
(e) Two genes, lan B and Lan C (or in some cases only one gene, Lan M), with no sequence similarity to other known gens thought to encode enzymes involved in the formation of lanthionine and methyllanthionine, and
(f) Two genes lan k and lan R encoding two component regulatory proteins that transmit an extracellular signal and thereby inducing lantibiotic production.

Each gene cluster appears to contain all the genes necessary for translation and post-translational modifications, when necessary, of a prebacteriocin, secretion and removal of the leader peptide, and self-immunity. (Ray et al., 2001).

The promoter upstream of the nis A gene is activated by the nis R and nis K proteins which induce transcription of the gene cluster. (de vos et al., 1995). Following translation of prerinisin, the leader peptide directs the precursor to the membrane located nis B protein that dehydrates serine to dhA and threonine to dhB. Nis C, which forms a complex with nis B, then forms thioether linkages between dehydration residues and cysteines (Ray et al., 2001).

The modifying enzymes, nis B and nis C are encoded directly downstream of nis A. These enzymes act on the prepeptide, modifying only the mature protein, which is then transported (Allison and Klaenhammer, 1999). Nis T encodes a protein that shares significant homology with ATP-dependent translator proteins, and is involved in the translocation of fully modified precursor nisin across the cytoplasmic membrane (Qiao and Saris,
Once outside the membrane, the leader peptide is removed from the biologically active precursor by the nis P which is an extracellular serine protease. With the removal of leader peptide, the matured pronisin (or nisin) molecule becomes biologically active and is released into the environment. Nis I encode a lipoprotein that is involved in immunity. Proteins F, E, and G also provide cells with additional protection against nisin. (Bhunia and Johnson, 1991; Motlagh and Ray, 1994; Bukhtiyarova and Yang, 1994; Ray and Miller, 2000).

2.4.11 Storage studies: - Gandhi and Nambudripad, (1981) reported that crude and partially purified antibiotic from *Lactobacillus acidophilus* was stored at -25°C for 6 months without any loss of activity. While lactacin B was stable during storage at room temperature or at -20°C for several months (Barefoot and Klaenhammer, 1984) without any loss of activity. ten-Brink *et al.* (1994) found that filter sterilized culture supernatant fluids containing acidocin B could stored at -20°C or 4°C for at least 90 days without loss of acidocin B activity while during storage at 37°C some inactivation occurred, possibly caused by the action of proteolytic enzymes present in culture supernatant. Dave and Shah (1997) reported that acidophilicin LA-1 was stable for >15 days at 37°C, >3 months at 4°C and >8 months at -18°C.

2.4.12 Applications: - The single most important reason behind the recent interest in isolating bacteriocin producing lactic acid bacteria and in studying bactericidal effectiveness of bacteriocin is their potential applications as food biopreservations (Ray *et al.*, 2001).

2.4.12.1 Food applications: - Food processors face a major challenge in an environment in which consumers demand safe foods with a long shelf life, but also express a preference for minimally processed products that do not contain chemical preservatives. Bacteriocins are an attractive option that could provide at least part of the solution. They are produced by food-grade
organisms, they are usually heat stable and they can inhibit many of the primary pathogenic and spoilage organisms that cause problems in minimally processed foodstuffs, however, at present, only nisin and pediocin PAI/AcH have found widespread use in food. The form of nisin used most widely in food is Nisaplin (Danis co), which is a preparation that contains 2.5% nisin with NaCl (77.5%) and non-fat dried milk (12% protein and 6% carbohydrate). The use of pediocin PAI for food biopreservation has also been commercially exploited in the form of ALTA 2431 (Quest), which is based on LAB fermentates generated from a pediocin PAI-producing strain of Pediococcus acidilactici (Rodriguez et al., 2002). Its use is covered by several US and European patents (Ennahar et al., 2000b; Rodriguez et al., 2002) when screening for a bacteriocin With a food application in mind, there are several important criteria: first, the producing strain should preferably have 'generally recognized as safe' (GRAS) status; and second, the bacteriocin should have a broad spectrum of inhibition that includes pathogens, or have activity against a particular pathogen. Third, the bacteriocin should be heat stable; fourth, have no associated health risks; fifth, its inclusion in products should lead to beneficial effects such as improved safety, quality and flavour; and sixth, it should have high specific activity (Holzapfel et al., 1995). Bacteriocins have been shown to have potential in the biopreservation of meat, dairy products, canned food, fish, alcoholic beverages, salads, egg products, high-moisture bakery products, and fermented vegetables, either alone, in combination with other methods of preservation, or through their incorporation into packaging film/food surfaces (Sullivan et al., 2002; Ryan et al., 2002; Chen and Hoover, 2003).

Although, bacteriocins with a wide spectrum of activity are usually the most sought after, other factors including pH optima, solubility and stability are as important and are major considerations in choosing a particular inhibitor for a particular food or target bacterium. Furthermore, the antimicrobial spectra of a variety of LAB bacteriocins can be extended to encompass Gram-negative bacteria through their use in combination with measures that affect the
integrity of the outer membrane, such as temperature shock, high pressure, chelators and eukaryotic antimicrobial peptides (Stevens et al., 1991; Delves-Broughton et al., 1996; Kalchayanand et al., 1998; Suma et al., 1998; Boziaris and Adams, 2000; Boziaris and Adams, 2001; Masschalk et al., 2003; Loders et al 2003; Baker et al., 2004). There are also rare natural [for example, AS48 (Abriouel et al., 2003)] and bioengineered bacteriocins (Yuan et al. 2004) that possess inherent activity against Gram-negative microorganisms.

Bacteriocins can also be used to promote quality, rather than simply to prevent spoilage or safety problems. For example, bacteriocins can be used to control adventitious non-starter flora such as non-starter lactic acid bacteria (NSLAB) in cheese and wine. The uncontrolled growth of NSLAB can cause major economic losses owing to calcium-D-lactate formation (Thomas and Crow, 1983) and slit defects in cheeses, and the production of detrimental compounds in wine. Bacteriocins producing starters and adjuncts (one- or two-strain strategies) have been found to significantly reduce these problems (Kadler, 1990; Daeschel et al., 1991, Ryan et al., 1990; Oumer et al. 2001; Sullivan et al., 2003;). However, as some NSLAB such, as lactobacilli and other starter adjuncts in cheese, and Leuconostoc oenos and Pediococcus damnosus in some red wines can improve flavour, the complete elimination of NSLAB is not always desirable (Fox et al., 1998). This problem has been overcome through the use of a three-strain system in which an adjunct strain with reduced bacteriocin sensitivity (obtained on repeated exposure to increasing concentrations of the bacteriocin) is used with a bacteriocin-producing starter (Ryan et al., 2001) Figure 9.

Bacteriocins can also be applied in other ways to enhance food fermentation. This has been shown during semi-hard and hard cheese manufacture in which bacteriocin production brings about the controlled lysis of starter LAB, which results in the release of intracellular enzymes and ultimately accelerated ripening and even improved flavour (Oumer et al., 2001; Martinez-Cuesta et al., 2002; Grade et al., 2002; Sullivan et al., 2003).
Although traditionally, the use of bacteriocins is associated with the preservation of food, in the near future food might merely act as a vehicle for the delivery of bacteriocin-producing probiotic bacteria. The production of antimicrobials by a probiotic culture is a desirable trait as they are thought to contribute to the inhibition of pathogenic bacteria in the gut (Marteau and Rambaud, 1993; Tannok, 1997; Dunne et al., 1999), whereas bacteriocins in food are degraded by the proteolytic enzymes of the stomach, probiotic bacteria might be ingested in a form that facilitates gastric transit, allowing the in vivo production of the bacteriocin in the small or large intestine. It has also been speculated that recombinant probiotic strains that can be induced to produce bacteriolysin could be developed to facilitate the in vivo delivery of bioactive compounds that are produced intracellularly (Hickey et al., 2004).

Three approaches are commonly used in the application of bacteriocins for biopreservation of foods (Schillinger et al., 1996):

1) Inoculation of food with LAB that produces bacteriocin in the products. The ability of the LAB to grow and produce bacteriocin in the products is crucial for its successful use.

2) Addition of purified or semi-purified bacteriocins as food preservatives.

3) Use of a product previously fermented with a bacteriocin-producing strain as an ingredient in food processing.

Unlike most other preservation methods, such as heat or low pH, which are essentially indiscriminate in their antimicrobial effect, it is this ability to precisely influence the developing flora in an otherwise perishable food that led us to describe the use of bacteriocins as a form of 'innate immunity' for food. As already described, the inclusion of Listeria-active class IIa bacteriocins can specifically prevent the growth of this pathogen, without affecting harmless LAB, or bacteriocin-tolerant strains can be introduced into an otherwise hostile food environment. It is unlikely that the use of bacteriocins in food will negatively impact on the natural flora of either the human (or animal) host, or on the environment. The low level of bacteriocins required eliminating or reducing small numbers of pathogenic or spoilage organisms in
food are unlikely to have an impact on more microorganism-rich environments. In any event, bacteriocins are unlikely to survive gastric transit, as they are sensitive to proteolytic degradation.

2.4.12.2 Clinical applications:—In particular, the elucidation of the precise mechanism of action of some lantibiotics and their activity against multidrug resistant pathogens by a novel mechanism makes them an attractive option as possible therapeutic agents.

The broad-spectrum lantibiotics could theoretically be of use against any clinical Gram-positive human or animal pathogen. For example, the two-peptide lantibiotic lacticin 3147 has \textit{in vitro} activity against \textit{Staphylococcus aureus} [including methicillin-resistant \textit{S. aureus} (MRSA)], enterococci (including VRE), streptococci (\textit{S. pneumoniae}, \textit{Streptococcus pyogenes}, \textit{Streptococcus agalactiae}, \textit{Streptococcus dysgalactiae}, \textit{Streptococcus uberis}, \textit{Streptococcus mutans}), \textit{Clostridium botulinum}, and \textit{Propionibacterium acnes} (Galvin \textit{et al.}, 1999). Initial \textit{in vivo} trials with animal models have demonstrated the success of lantibiotics in treating infections caused by \textit{S. pneumoniae} (Goldstein \textit{et al.}, 1998), and MRSA (Niu and Neu, 1991; Kruzewska \textit{et al.}, 2004), and in preventing tooth decay and gingivitis (Howell \textit{et al.} 1993; Blackburn and Goldstein, 1995; McConville, 1995; Patel, 1995; Ryan \textit{et al.}, 1999).

The use of nisin for human clinical applications has been licensed to Biosynexus Incorporated by Nutrition 21 and Immucell Corporation has licensed the use of the anti-mastitic nisin-containing product Mast Out to Pfizer Animal Health. \textit{Bovine mastitis} is defined as an inflammation of the udder and is the most persistent disease in dairy cows. Nisin is also used as an active agent in WipeOut (a teat wipe), and lacticin-3147-containing Teat Seals (Cross VetPharm Group Ltd) have been shown to prevent deliberate infection by mastitic staphylococci and streptococci in animal challenge trials (Ryan \textit{et al.}, 1999) Figure 8.
Figure 9: Selected applications of bacteriocins. a | Food quality. A cheese made with a commercial starter culture (Bac –) will develop an undefined flora called non-starter lactic acid bacteria (typified by different fingerprints generated by random amplified polymorphic DNA patterns). However, a cheese inoculated with the same commercial strain that can produce a bacteriocin (Bac +) (lacticin 3147, in this example) and a resistant adjunct strain of Lactobacillus, chosen for a flavour attribute, will develop a single defined culture once the starter culture has died off, offering the cheese manufacturer control over previously adventitious flora development. b | Food safety. A simple example of the role of bacteriocins in food safety is the production of cottage cheese with a starter culture that produces a bacteriocin with activity against Listeria monocytogenes, which results in a cheese that is inherently anti-Listeria. c | Veterinary medicine. A test seal is a physical barrier against infection. Here, a bacteriocin was incorporated into the test seal and the test was challenged with Staphylococcus aureus. The number of staphylococci recovered from 14 teats with or without bacteriocin is shown. d | Human medicine. A Streptococcus mutans strain that cannot produce acid, but that produces the lantibiotic mutacin (shown), can competitively exclude acidogenic S. mutans, thereby offering protection against tooth decay (Hilman, 2002).

Source:– Cotter et al. (2005).

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Animal Health. *Bovine mastitis* is defined as an inflammation of the udder and is the most persistent disease in dairy cows. Nisin is also used as an active agent in WipeOut (a teat wipe), and lacticin-3147-containing Teat Seals (Cross Vetpharm Group Ltd) have been shown to prevent deliberate infection by mastitic staphylococci and streptococci in animal challenge trials (Ryan *et al.*, 1999) Figure 8. A strain that produces the lantibiotic mutacin 1140 is entering Phase I clinical trials in the US with a view to replacement therapy, and the dietary supplement BUS K12 throat guard, which contains a *Streptococcus salivarius* that produces two lantibiotics salivaricin A2 and B, is sold in New Zealand as an inhibitor of the bacteria responsible for bad breath (Tagg, 2004). From a nonantimicrobial medical perspective, the cinnamycin-like lantibiotics have attracted interest owing to their novel activities against the functions of medically important specific human enzymes, such as phospholipase A2 and angiotensin-converting enzyme, and nisin has also been found to have contraceptive efficacy (Aranha *et al.*, 2004; Reddy *et al.*, 2004). The effectiveness of probiotics as agents in the treatment of various gastrointestinal disorders has also been shown in several recent studies (Fedorak and Madsen, 2004).

For commercial and industrial reasons, the selected probiotics and starter cultures must be produced under the most stringent fermentation and manufacturing conditions. In general, the selection criteria for starter cultures are at their acidification rate and flavour-producing characteristics. For probiotics, selection is based on a detectable health effect on the host. Subsequently, the production of starter cultures and probiotic cultures is optimized to achieve greater biomass with high viable counts, improved stress survival during drying, and prolonged shelflife (Prasad *et al.*, 2003; Corcoran *et al.*, 2004). In addition, the use of low-cost industrial growth substrates and microbial strains with low phage sensitivity are regularly considered as important objectives for both strain and process improvement. Most of these
strain and process-improvement strategies are based on screening and trial-and-error approaches.

Genome-sequencing and functional-genomics studies that focus on LAB which are used as part of industrial starter cultures (Kleerebezem et al., 2003) or exploited for their probiotic properties (Altermann et al., 2005) are rapidly revealing the molecular basis of relevant traits, including stress-response-adaptation mechanisms and amino-acid and vitamin auxotrophies (Siezen et al., 2004).

Some LAB secretes vitamins, including riboflavin (vitamin B_2_), folate (vitamin B_12_) and cyanocobalamine (vitamin B_12_2) (Taranto et al., 2003). This unique characteristic offers the food industry the possibility to fortify raw food materials such as soy, milk, meat and vegetables with B vitamins without adding food supplements (Kleerebezem and Hugenholtz, 2003). Currently, in the fermentation industry, starter cultures are selected on the basis that they can produce and secrete high levels of vitamins B. In addition to natural strain selection, overproduction of vitamin B_2_ and vitamin B_12_ has been achieved by genetic engineering of the corresponding biosynthesis pathways of L. lactis (Sybesma et al., 2003; Burgess et al., 2004; Sybesma et al., 2004). However, metabolic engineering of such complex biosynthesis pathways can often lead to unexpected phenotypes because the products, being co-factors in various biochemical reactions, impact directly on many other pathways. LAB are also good candidates for the production and delivery of heterologous proteins and peptides that have potential therapeutic activity (Miyoshi et al., 2002; Loir et al., 2005; Mierau et al., 2005a; Mierau et al., 2005b). Many LABs are acid and bile resistant, and are therefore, well adapted to function as vehicles for the oral delivery of vaccine antigens (Mercenier et al., 2000). The best-studied LAB used, as vaccine vectors are Lactococcus lactis and Lactobacillus plantarum. Robinson et al. (1997) showed that intragastric or intranasal administration of recombinant lactococci expressing tetanus toxin fragment C resulted in the induction of systemic antibody responses in mice at levels sufficient to be protective against a lethal challenge with tetanus toxin.
Lactococcal immunization was also found to induce mixed immunoglobulin-G and T-helper responses, allowing the induction of protection against various infectious agents and potentially at several mucosal surfaces (Robinson et al., 2004). The impact of overproduction of heterologous proteins on the lactococcal host cells can be significant, as evident from the general stress response usually associated with protein overproduction (Schweder et al., 2002). In addition, the drain on amino acids used in heterologous protein biosynthesis can cause global effects on the metabolism of the host cell.

Thus, keeping in view the wide application of bacteriocins in food industry and clinical microbiology, it was felt desirable to undertake the present studies with an aim to isolate and characterize high bacteriocin producing strains of LAB from milk and milk products. It was also aimed to standardize various parameters for cheaper and optimum production of bacteriocin from the selected strain. The effects have also been made to purify and identify the bacteriocin during the present investigation.

The present study has been undertaken with the following objectives-
1. Bacteriocin producer was isolated from various food products available in the market.
2. The isolated strains screened for the production high concentration of bacteriocin.
3. To characterized the high bacteriocin producing strain by morphological, physiological and biochemical methods.
4. To check the preliminary antagonism of the strains against different bacterial species.
5. To prepare the cell free filtrate for testing the inhibitory activity.
6. To detect the presence of bacteriocin using microdilution well plate methods.
7. To make partial purification of bacteriocin and measure its activity.
8. To analyse the amino acid sequence of the purified preparation.
9. To find out the possibility of using the bacteriocin in the protection of food.