1.1 INTRODUCTION

Lactic acid bacteria have been used by man as probiotics, preservative and producer of fermented food since the start of human kind. They have produced some important metabolites like acids, alcohols, diacets and some antibiotics like inhibitory proteinaceous molecules commonly called as bacteriocins (Szkaradkiewicz and Karpinski, 2013; Klaenhammer, 1986). Bacteriocins are ribosomally synthesized antimicrobial peptide or complex proteins (carbohydrate or lipid associated) secreted by diverse class of Gram-positive or Gram-negative bacteria (Desriac et al., 2010).

The bacteriocin was found active against closely related bacterial species growing in the same ecological niche. These also have been found active against human and animal microbial pathogens (Cintas et al., 2001). It was previously reported that these peptide antibiotics have wide therapeutic potentialities, significant physico-chemical stability and no toxicity to human (Nagao et al., 2009). Therefore, the potentialities of bacteriocins have been well explored in the field of clinical microbiology, diagnostic microbiology, agriculture and veterinary. Many bacteriocins have been isolated, purified and characterized from a number of Lactobacillus species. However, other genera of lactic acid bacteria like Carnobacterium, Enterococcus, Lactococcus, Leuconostoc, Pediococcus, and Streptococcus (Hoover and Steenson, 1993) have also been reported to produce bacteriocin of different classes but members of these groups may be pathogenic.

Bacteriocin producer bacterial strain protects themselves against the toxicity of their own bacteriocins through the production of a specific immunity protein, generally encoded by bacteriocin operon. Besides the chemicals or antibiotics, the numerous natural antimicrobials molecules have been reported but most of them, have not shown satisfactory activity required for commercial development. However, some natural derived antimicrobial
molecules like bacteriocins, have been reported with significant broad
spectrum activity against infectious organisms and nisin like bacteriocins
have been commercialized in different formulations, multi-hurdle preservative
systems and therapeutic system (Paul et al., 2013).

A number of bacteriocins have been tested to control microbial growth
not only in food and beverage products, but also in plant and animal
protection. Bacteriocins like lacticin 3147 (Rea et al., 2007) and nisin A, have
been used to control drug resistance pathogens like S. aureus (MRSA) and
Vancomycin Resistant Enterococci (VRE). (Piper et al., 2009, 2011). The
inhibitory effects on various infectious organisms H. influenza, M.
catarrhalis, S. aureus, Enterobacteriaceae, P aeruginosa. S. pyogenes,
Neisseria meningitidis, Pasteurella multocida, H. Influenza and
Mycobacterium tuberculosis by bacteriocin have also been reported (Mandell
et al., 2007). The dermal absorption of broad spectrum bacteriocin,
Gallidermin, in rats has been successfully evaluated against P. acnes and S.
aureus (Manosroi et al., 2010).

Besides these, bacteriocins have been reported beneficial in dental
infection, vaginosis, spermicidal or contraceptive, antiviral and as or
probiotics of intestinal protector, which can be supplement with antibiotics
therapy. The preferred uses of bacteriocins among the consumer have been
increased in many applications due to their physically stability, non toxic
behavior and activity at nanomolar concentration (Sheila et al., 2005; Morgan
et al., 2005). Moreover, thermal processing is carried out extensively in food
processing and required physical stability of additives, which is also a
desirable criterion to formulate an active pharmaceutical ingredient into
suitable dosage forms. Thus, bacteriocins have been found stable at various
physico-chemical conditions and become the choice of alternative
preservative or therapeutics in many preparation. However, Stabilization,
increasing potency and shifting antimicrobial spectrum of bacteriocin, still a challenging field. To stabilize or to increase the potency of bacteriocins, biological and chemical modifications are needed to be revolutionized. For this, the scientists have used modern techniques of genetic engineering to produce engineered bacteriocin and many researchers have been accelerated in this field. However, pharmacokinetics and pharmakodynamic behaviors of bacteriocins are not much reported and more investigations are still needed for the further development of bacteriocins as an alternative therapeutic molecule in the field of medicines.

The bacteriocins suitability in pharmaceuticals has been presented through the analysis of cytotoxicity, inhibitory effects on the natural micro biota, and in vivo efficacy in mouse/rat models. Furthermore, a serious threat of antibacterial resistance has been reduced by use of nisin like bacteriocin but to fight against infectious and multidrug resistance pathogens, there is always a need of novel bacteriocin. Recently published data on bacteriocins or engineered bacteriocins, suggested that bacteriocins of different classes could be an alternative means of controlling such pathogens.

Thus, based on the significant potentialities of bacteriocin in the diverse field, the present study was designed in search of a potential bacteriocin, from novel bacterial strain that could be used as an alternative antibiotic against infectious bacterial/ fungal pathogens or an additive in food preservations.

1.2 RESEARCH OBJECTIVES

LAB (Lactic Acid Bacteria) is generally regarded as safe (GRAS) and forms a major group of probiotics bacteria producing a large numbers of useful metabolites. Members of genus Lactobacillus are reported as safe and beneficial bacteriocin producing organisms while other genera like Streptococcus and Enterococcus, contain many opportunistic pathogens. Thus, in search of novel bacteriocin, this study is targeted to isolate, purify
and characterize a bacteriocin from safe genera of lactic acid bacteria, e.g. \textit{Lactobacilli}. Therefore, the objectives of the proposed research are -

- To isolate strains of LAB from food or clinical samples and screen them for their abilities to produce bacteriocins against pathogenic and food spoilage bacteria and fungi.
- To characterize the selected bacteriocin producing \textit{Lactobacilli} based on morphological, physiological and biochemical methods.
- To characterize the selected bacteriocin producing \textit{Lactobacilli} based on molecular methods and phylogenetic analysis.
- To optimize culture conditions of selected isolate and optimize the nutrient components for efficient production of bacteriocin.
- To purify and characterize the bacteriocin from selected isolate

1.3 HYPOTHESIS AND SIGNIFICANCE OF THE STUDY

Bacteriocins are ribosomally synthesized, a functionally distinct family of antimicrobial peptides or proteins, produced by various bacteria, fungi, plants and animals. It is widely accepted by consumers as preservative or therapeutic agent, because of its pretentious, stable and non toxic nature. The area of bacteriocins has been successfully explored in the field of clinical microbiology. It has been widely tested in the area of food, agriculture and health sectors, particularly to control drug resistance pathogens of human as well as animals. The genetic engineering is creating a revolution constantly, in the study and designing of stable bacteriocin, with increased efficacy or antimicrobial spectrum. Based upon some physico-chemical properties and broader antimicrobial spectrum of previously described bacteriocin by researchers indicate that the use of bacteriocin could be advantageous over currently used antibiotics, not only against sensitive pathogens, but also
against various drug resistance microbial pathogens. The previously reported bacteriocin from genus *Bacillus* might be a potential source to control microbial pathogens and reduce the emerging problems of drug resistance among a number of microbial pathogens, developed from the frequent use of antibiotics. Therefore, these peptide antibiotics could be a significant source to treat the various diseases in the future.

The bacteriocin from various *Bacillus* of order fermicutes have been isolated, purified, characterized and tested against many microbial pathogens. The *Lysinibacillus* also belong to order fermicutes and it has been isolated from soil, water and various foods or clinical samples. Some species have been described as boron tolerant and insecticidal. However, the antimicrobial potential against bacterial and fungal microbial pathogens has not yet been evaluated. Thus, it is pertinent to conduct the study on *Lysinibacillus* for its additional feature and inhibitory significance against fungal and bacterial pathogens. The isolated, purified molecule could be used in diagnosis, mitigation, curing and treatment of various infectious or cancers like diseases. Therefore, through this study, an attempt was made to characterize isolate on the basis of physico-chemical and molecular characters. We also explored the inhibitory significance of isolated strains of *Lysinibacillus* against many foodborne bacterial/fungal pathogens. A purified bacteriocin from isolate 3 also has been evaluated for its inhibitory significance against *Bacillus pumilus*.

1.4 REVIEW OF LITERATURE

The Antibiotics of the twentieth century have emerged to decrease the mortality, drug resistance and morbidity among the human population, but these are costly and toxic to beneficial intestinal micro flora (White, 2011; Cooper and Shlaes, 2011; Blaser, 2011).
Numerous researches have been reported that effective alternative molecules are plant derived compounds (Savoia, 2012), bacteriophages and phage (Mc Conville and Enright, 2011; Burrowes et al., 2001), RNA-based therapeutics, probiotics, and antimicrobial proteinaceous molecule likes bacteriocin. Based upon some physico-chemical properties and broader antimicrobial spectrum of previously described bacteriocin by various researchers indicated that the use of bacteriocin could be advantageous over currently used antibiotics, not only against sensitive pathogens but also against various drug resistant microbial pathogens (Kole et al., 2012; Shanahan, 2010; Papagianni, 2003). Therefore, these peptide antibiotics could be a significant source to treat various diseases in the future (Li et al., 2012). The diversity of bacteriocins among producers is described in terms of size, molecular structure, biosynthesis, mode of action and the antimicrobial spectrum (Sharma et al., 2009; McAuliffe et al., 2001; Dicks et al., 2001). It was previously reported that these peptide antibiotics have high potentialities (Paul et al., 2013; Sheila et al., 2005), significant physico-chemical stability and no toxicity to human (Nagao, 2009).

Thus, various bacteriocins have been recovered for their remarkable potential as food preservative (Papagianni., 2003) or as therapeutic agents to control Gram -positive and Gram-negative infectious bacteria (Van Heel et al., 2011; Van Staden et al., 2012). The use of bacteriocin in agricultural field has also been signified as bio-controlling agents for plant and crop protection (Papagiani, 2003; Chatterjee et al., 1992).

1.4.1 Physico-chemical classification of bacteriocin

Gram-positive and Gram-negative bacteria have been determined to produce bacteriocins of different classes. The bacteriocin of Gram-positive bacteria can be ranked according to their biochemical, genetic characteristics or presence of disulphide or monosulphide bonds. Abee et al. (1994 b)
classified bacteriocin into four groups according to their molecular weight, heat stability, proteolytic enzyme stability, the presence or absence of post translational modified amino acids and antimicrobial action (Figure 1.1)

![Classification of bacteriocin based on physico-chemical properties](image)

**Figure 1.1**: Classification of bacteriocin based on physico-chemical properties

### 1.4.1.1 Class I bacteriocin

This class of bacteriocins included small peptides (< 5 kDa, 19-37 amino acids) and regarded as lantibiotics due to presence of unusual amino acids lanthionine and methyl lanthionine in their primary structure. Nearly sixty lantibiotics have been identified, out of which, 30% lantibiotics (20) are purified from LAB (Bierbaum and Sahl, 2009; Bonelli *et al*., 2006). These bacteriocins are heat-stable peptides and generally act by targeting the skeleton of the cell wall of sensitive pathogen particularly against Gram-positive (González-Martínez *et al*., 2003). These are comprehensively
postranslationally modified, changed to characteristic lanthionine and methyl lanthionine. Biosynthesis and biological activities of uniquely modified peptide has been isolated from Gram positive bacteria (Hans-George *et al*., 1988). The modification arises via a two-step process, catalyzed by two enzymes encoded by gene LANB and LANC. With gene LANB, serine and threonine of propeptide, are subjected to dehydration consequence, the formation of dehydroalanine and dehydrobutyrine, respectively (Sahl and Bierbaum, 1998).

The enzyme encoded by LANC, catalyze the formation of lanthionine and methyl lanthionine via, cyclization of cysteine on to DHa and DHb (Chatterjee *et al*., 2005). Mostly, only three amino acid of the peptide structure (serine, threonine and cysteine) undergone for post translational modification. However lysine, alanine and isoleucine may also be occasionally involved in post translational modification e.g. nisin. Although, lacticin 481, cytolysine and salivaricin are post translationally modified under one gene LANM only, encoded by an enzyme of 900-1000 amino acid residues responsible for both socialization and dehydration of the peptides. Thus, these lantibiotics formed a distinct group from the group of nisin like lantibiotics (Chatterjee *et al*., 2005; Hans-Georg and Bierbaum, 1988). Furthermore, from the observation of the structure of both groups’ bacteriocins, it was found that post translation modification involved in the formation of multiple thio ether rings in peptide structure to provide antimicrobial activity and also peptide stability, against proteolytic or thermal degradation (Sahl and Bierbaum, 1998; Cotter *et al*., 2005; McAuliffe *et al*., 2001). After synthesis and post translational modification, the peptide is transported to the exterior of the cells, through ABC transport system. Ladder peptide is processed by specific protease before or after transport and produce a mature, active peptide (McAuliffe *et al*., 2001). Thus, based on the
structural similarities, lantibiotic bacteriocins are initially divided into two subclasses.

1.4.1.1.1 Subclass IA

These peptides are positives charged elongated bacteriocins, that kill the sensitive microbial cells of bacterial species by pore formation. The prototypes antibiotic nisin is a fellow of this group.

1.4.1.1.2 Subclass IB

These bacteriocins like, lacticin 481, lacticin 481, cytolysine and salivaricin are characteristically globular, inflexible with negatively charged or without any net charge. They inhibit various catalytic enzymes required to complete the life supporting processes of the susceptible bacteria (Deegan et al., 2006).

1.4.1.2 Class II bacteriocins.

The bacteriocins belonging to this class are generally regarded as non-lantibiotics or non modified or pidocin like antibiotics. These are bacteriocins of varying molecular weight, small peptide usually made up of 37-49 amino acid residues, (<10 kDa), heat-stable, non lanthionine, membrane active peptides characterized by the presence of hydrophilic N terminal, consensus sequence TyeGlyAsnGlyValXaaCys.(YGNGV(X)C(X)4C(X)V(X)4A) where x is the any amino acid and a more varying hydrophobic or amphiphilic C terminus (Drider et al., 2006; Sparo, et al., 2006). Because of the earliest discovery and the presence of this consensus sequence in pidocin A1, these bacteriocins are also regarded as pidocins like bacteriocins. They have a high isoelectric point (PI 8-10), so found positively charged at physiological pH. These bacteriocins are also characterized by the presence of a self protective, transport signal receiver, cleavage site as double glycine motif and help the peptide to transport the exterior of the cell via ABC transport system (Van Belkum et al., 1997). However, bacteriocin 31 and enerocin P, lack of double
glycine motif in their peptide structure and transported by secretory dependent general secretory pathway (Cintas et al., 1997). This group of bacteriocin was further classified into three subgroups.

1.4.1.2.1 Class IIA

Antilisterial, bacteriocin are grouped in this class. The representative bacteriocins of this group are leucocin A, acidocin A (Ennahar et al., 2000) mesentericin pediocin PA-1 and sakacin P (Venema et al., 1997).

1.4.1.2.2 Class IIB

The bacteriocins of this group are protein complex, require at least two different peptides for activity and thus generally act synergistically. These peptides do not have much or no activity when they tested individually. However, these seem to be no sequence resemblance with each other. Lactococcin G and plantaricins (Gong et al., 2009) are representative bacteriocin of this group.

1.4.1.2.3 Class IIC

The bacteriocins of this class are small peptides, heat-stable, which are carried by leader-peptides. The bacteriocins of this class are further divided into 2 groups of bacteriocin, thiolbiotics and cystibiotics. Thiolbiotics are bacteriocin with two cysteine residues and with one cysteine residues are cystibiotics bacteriocins. Lactococcin A, divergicin A and acidocin B are bacteriocins of class IIc.

1.4.1.3 Class III bacteriocins

These bacteriocins are large peptides, with a molecular weight over 30 kDa. In this class, bacteriocins lie are zoocin A, lysostaphins helveticins J (Joerger and Klaenhammer, 1986) and helveticins V (Vaughan et al., 1992). Based upon lytic and non lytic nature, these bacteriocins are classified as heat labile lytic bacteriocin and heat labile non lytic bacteriocins. The lytic bacteriocins are generally endopeptidase peptide which lyses the cell wall of
sensitive bacteria in enzymatic manner. Literature on the structure of lytic bacteriocin; enterolysin A, Zoosin A and lysostaphins indicated that leader peptide is made up of 16 to 36 amino acids. The leader sequence of these shares a little sequence homology but all are characterized by the presence of a signal sequence. This signal sequence is involves in its transportation in the cell membrane via translocase complex under general secretory or Sec dependent secretory pathway.

The translocase complex consist of a motor domain protein sec A, the protein conducting channel formed by three proteins secE, sec Y, sec G and the accessory protein secD and sec F. The immature bacteriocin translocated to exterior of the cell via sec A, Sec Y, secE, and sec G respectively. The enzymatic cleavage of peptide signal and closing of sec channel result in transport of mature bacteriocins to the exterior of the cells. The target of lytic bacteriocin is the cell wall of Gram-positive bacteria. They cleave off specific peptide bond, the insoluble peptidoglycan layer is solubilized, resulting in a loss of integrity of the cell wall which cannot be longer withstand the cell internal turgor of up to 25 atmosphere. Thus, resulting in cell death via cell lysis of sensitive organism.

Beside lytic bacteriocin, some heat labile and high molecular weight bacteriocin without lytic mode of action have been found. Helveticin J, from L. helveticus 481 dysgalactici from Streptococcus dysgactiae spp. Equisimilis W2580 and strptococcin A-M57 are bacteriocin of this class. The mode of action dysgalacticin has been studied and it was concluded that this bacteriocin interfere either glucose transport or metabolism by binding with phosphoenol-pyruvate-dependent-glucose and mannose phosphotransferase transport system within the cell membrane sensitive cells.
1.4.1.4 *Class IV or complex bacteriocins or cyclic bacteriocin*

These peptides are involved in lipid or carbohydrate moiety thus these bacteriocins are regarded as complex bacteriocins. Due to presence of carbohydrate or lipid moiety, these bacteriocins are sensitive to glycolytic or lipolytic enzymes. Plantaricin S and leuconocin S are bacteriocins of this group (Lewus and Montville, 1991). Recently, a novel cyclic Uberolysin like bacteriocin secreted by *Streptococcus uberis* has also been described (Wirawan *et al.*, 2007).

1.4.2 *Structural classification of bacteriocin*

Consistently, molecular weight and physico-biochemical properties of bacteriocins are utilized to group the bacteriocins. However, these systems of bacteriocin classification lack consistency and coherence because many bacteriocins belonging to more than one class at the same time. Therefore, these bacteriocins cannot be classified into above described distinct class of classification. Therefore, in the light of structural data, Zouhir *et al.* (2010) proposed a new structure-based sequence fingerprint classification of bacteriocins into 12 groups (Figure 1.2). This system provided a consistent pattern of bacteriocin classification even to classify the unclassified bacteriocin and helpful in classifying more than 70% known bacteriocin to date. The identified groups are distinguished by the occurrence of extremely conserved sequences of amino acid motifs. The twelve groups of bacteriocin of this classification are briefly outlined in the table 1.1.
Figure 1.2: Structural classification of bacteriocin (Zouhir et al., 2010).
<table>
<thead>
<tr>
<th>Class</th>
<th>Consensus sequence</th>
<th>p value</th>
<th>Example</th>
</tr>
</thead>
<tbody>
<tr>
<td>Class I</td>
<td>GX3TX3EC</td>
<td>7.06×10^{-18} &lt; p-value &lt; 3.08 ×10^{-08}</td>
<td>mutacin 2, lacticin 481, bacteriocin J46, ruminococcin A, variacin, lacticin 3147, mersacidin, actargadine, plantaricin W alpha, nukacin ISK1, mutacin H29B, streptococcin AFF22, and streptococcin AM49</td>
</tr>
<tr>
<td>Class IA</td>
<td>GXXCTLXXEC</td>
<td>5.19×10^{-13} &lt; p-value &lt; 6.11 ×10^{-11}</td>
<td>Lacticin 3147 A1, actargadine, plantaricin, W alpha and mersacidin</td>
</tr>
<tr>
<td>Class IB</td>
<td>GVX2TXSH/ YECX2NS/TW/FQ/AF/HV/LA /FTCC</td>
<td>5.37×10^{-24} &lt; p-value &lt; 1.25 ×10^{-18}</td>
<td>Mutacin 2, bacteriocin J46, lacticin 481, ruminococcin A, streptococcin AM49, nukacin ISK1, streptococcin AFF22, mutacin H29B.</td>
</tr>
<tr>
<td>Group 2</td>
<td>CX2SCSXGPXTX2CDGNTK</td>
<td>1.81×10^{-23} and 1.27 ×10^{-19}</td>
<td>Duramycin C, cinnamycin and ancovenin</td>
</tr>
<tr>
<td>Group 3</td>
<td>KYYGNGVXCKX2CXVD/NWX2A</td>
<td>3.06×10^{-22} and 1.73×10^{-16}</td>
<td>bacteriocin 31, Lactococcin MMFII, enterocin SEK4, divergicin M35, bavaricin MN, enterocin A, divercin V41, carnobacteriocin BM1, piscicocin V1b, curvacin A, enterocin P and sakacin A</td>
</tr>
<tr>
<td>---------</td>
<td>------------------------</td>
<td>---------------------------------</td>
<td>----------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Group 3 a</td>
<td>YGNVYCNX2KCWX8I</td>
<td>1.36×10^{-23} &lt;p-value&lt;2.4×10^{-18}</td>
<td>Lactococcin MMFII, bacteriocin31, enterocin SEK4, divergicin M35, enterocin A, bavaricin MN, divercin V41, carnobacteriocin BM1, piscicocin V1b, enterocin P, curvacin A and sakacin A</td>
</tr>
<tr>
<td>Group 3 b</td>
<td>KYYGNGVXCKX2CWXW</td>
<td>1.14×10^{-14} and 4.95×10^{-11}</td>
<td>Carnobacteriocin B2, leucocinC, listeriocin 743A, bavaricin A, sakacin P, mundticin,enterocin CR35, mundticin KS, piscicolin 126, piscicocinV1a,</td>
</tr>
</tbody>
</table>
### CHAPTER 1

<table>
<thead>
<tr>
<th>Group</th>
<th>Sequence</th>
<th>P-value</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>SX3CTPGC</td>
<td>$1.3 \times 10^{-17}$</td>
<td>coagulin A, pediocin PA1, mesentericin Y105, leucocin A, leucocin B Talla, plantaricin C19, and prebacteriocin423</td>
</tr>
<tr>
<td>4a</td>
<td>SXSLCTPGCXTGALX2CX3T XTCXI</td>
<td>$2.84 \times 10^{-36}$</td>
<td>Streptin, epidermin, gallidermin, mutacin 1140, mutacin B Ny 266, subtilin, nisin A, and nisin Z</td>
</tr>
<tr>
<td>4b</td>
<td>SX3CTPGCAXTGSFNSYCC</td>
<td>$5.41 \times 10^{-14}$</td>
<td>subtilin, nisin A, nisin Z</td>
</tr>
<tr>
<td>5</td>
<td>KATRX2TVSCK</td>
<td>$7.65 \times 10^{-11}$</td>
<td>Streptin, epidermin, gallidermin, mutacin 1140, mutacin B Ny 266</td>
</tr>
<tr>
<td>6</td>
<td>SX3CPTTKC X3C</td>
<td>$2.5 \times 10^{-7}$</td>
<td>Pep 5, epicidin 280)</td>
</tr>
<tr>
<td>7</td>
<td>VX2CAS</td>
<td>$5.08 \times 10^{-10}$</td>
<td>plantaricin W beta, Cytolysin, and lacticin 3147 A2</td>
</tr>
</tbody>
</table>

1132 beta, plantaricin 1.25 beta and Acidocin J
| Group 8 | GX3  
GGLX2IPXGPLXWXAGXAXV | $1.09 \times 10^{-9}$ <p-value$<1.09 \times 10^{-10}$ | Carnobacteriocin A and enterocin B |
<table>
<thead>
<tr>
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</tr>
</thead>
<tbody>
<tr>
<td>Group 9</td>
<td>CGPACX3GAHYXPIWX2VTAATGGFGBKIRK</td>
<td>$1.17 \times 10^{-34}$ &lt;p-value$&lt;2.08 \times 10^{-34}$</td>
<td>Plantaricin S alpha, lacticin F, gassericin T.</td>
</tr>
<tr>
<td>Group 10</td>
<td>GXWGX6GGAAXGGX2GY</td>
<td>$1.04 \times 10^{-25}$ &lt;p-value$&lt;9.73 \times 10^{-15}$</td>
<td>Lacticin F lafX, acidocin LF 221B and lactobin A</td>
</tr>
<tr>
<td>Group 11</td>
<td>GX2GX2QX3DFX2GX3GI</td>
<td>$1.67 \times 10^{-7}$ and $4.94 \times 10^{-7}$</td>
<td>Lactocin 705 and divergicin 750</td>
</tr>
<tr>
<td>Group 12</td>
<td>WX9GX3G</td>
<td>$1.02 \times 10^{-7}$ &lt;p-value$&lt;7.01 \times 10^{-6}$</td>
<td>Lactococcin G beta, sublancin 168 and PlnJ</td>
</tr>
</tbody>
</table>
1.4.3 **Properties and advantages of bacteriocins**

From the literature analysis of previously conducted researches, most of the bacteriocins ranged from the low molecular weight bacteriocin to high molecular weight bacteriocin which nearly lies from 2 kDa to 300 kDa. The low molecular weight bacteriocin of class II including lantibiotics and non-lantibiotics, are characterized by high isoelectric point, highly cationic nature at pH 7 and having a hydrophobic and hydrophilic residues (Jack *et al*., 1995; Nes *et al*., 1996). These bacteriocins are active at pH 3-7, which may reflect the adaptation of these substances to the environmental conditions in which bacteriocin-producing organism growing (Cintas *et al*., 1997). The optimum solubility and stability of these bacteriocins have been observed in an acidic environment (pH 2-6) (Figure 1.3). However, irreversible inactivation of these bacteriocins has also been observed at neutral pH (Hurst, 1981).

This nature has been found with previously described bacteriocins like Lactostrepcins (Kozak *et al*., 1978) Plantaricin C (Gonzáleze *et al*., 1994), leucocin A-UAL-87 and enterocin 1146, enterocin A (Parente and Hill, 1992). On the other hand some bacteriocin of high molecular mass 30–300 kDa appears as large aggregates in their native state. The native state of bacteriocin as aggregates shows antimicrobial activity while the dissociated form might be pretense partially or completely the inhibitory potentialities of the antimicrobial peptide. This is not only related to loss of activity, but also in the purification and creating the difficulties in the determination of exact molecular weight. The disaggregation of bacteriocin is due to their non polar nature or exposed to the bacteriocin to harsh environment like contact with SDS, sonication etc, resulting decrease or loss of bacteriocin activity. That is why, care should be taken or such techniques in isolation and purification should be avoided. However, to recover such type of bacteriocin it is desirable to concentrate the source material. Therefore, the recovery or purification of
such type of bacteriocin, vacuum drying or lyophilization is recommended (Cintas et al., 2001). However, bacteriocin therapy may also suffer from some disadvantages like development of resistance (Cotter et al., 2005), sensitivity to proteases which did not permit its longer duration of action. However, longer duration of action can be achieved by usage of specialized drug delivery systems or peptide modification in the form of dendrimers (Tam et al., 2002, Bracci et al., 2003 Nigama et al., 2014) that may prevent degradation and also permit safe delivery of them at the site of infection.

![Properties of bacteriocins](image)

**Figure 1.3: Properties of bacteriocins**

### 1.4.4 Mechanism of action of bacteriocins

Different classes of bacteriocin have been shown to exert their action by diverse means. They act either by lipid or surface molecular binding, specific- nonspecific receptor binding or by direct cell wall mediated cell
lysis, resulting in cell death via dissipation of the proton motive force, cell wall synthesis inhibition or DNA/protein synthesis inhibition of susceptible cells (Chikindas et al., 1993, Montville and Chen, 1998). Many cell surfaces interacting molecules like mannose-phosphotransferase (man-PTS) system or lipid II molecules have been recognized as interacting molecule with class I bacteriocin. Lipid II, involved in cell wall synthesis and serves as an anchoring receptor for the binding of vancomycin/teicoplanin family of glycopeptides, mannopeptimycins, ramoplanins, katanosins/plusbacins and lantibiotics (Clardy et al., 2006) (Figure 1.4).

Subclass Ib antimicrobial peptides are distinguished on the basis of rigid and globular nature, inhibiting DNA/RNA/protein or cell wall synthesis of sensitive bacteria (Deegan et al., 2006). The bacteriocins of class IIa, target an evolutionary well known subgroup of man-PTS on responsive cells of genera such as Listeria, Enterococcus (Kjos, et al., 2009). Thus, these bacteriocins interacted with the target cell by variable mechanism, even also electrostatic binding to the membrane and/or specific to a membrane-associated component (Abee et al., 1994b; Hammami et al., 2013). For example, the N-terminal domain of class II bacteriocins plays a significant role in electrostatic binding to the membrane surface of targeted pathogens, while the C-terminal region is important for determining target specificity (Fimland et al., 1996). However, different model such as barrel-stave model, the carpet model and the wedge model, have also been proposed in support of pore formation which is facilitated by direct or indirect cytoplasm translocation of bacteriocin. The interaction of proteinaceous membrane components in mechanism of action was first time described by Chikindas et al., (1993), using protease treated membrane vesicles, derived from sensitive cells, for pediocin PA-1. This hypothesis was further supported by prerequisite of a chiral interface for leucocin A activity (Manilduth et al.,
Lacticin 3147 bind to lipid components of the cells of bacteria and followed by insertion of the more linear LtnA2 peptide into the target membrane, resulting in pore formation and ultimate cell death. The peptidoglycan biosynthesis inhibition mediated by lipid II, has also been reported with bacteriocin LtnA1.

**Figure 1.4:** Mechanism of action of bacteriocins

### 1.4.5 Synergistic action of bacteriocin

Previously, it was reported that many bacteriocin have shown synergistic action with antibiotics or plant derived compounds. Ettayebi *et al.*, 2000 has reported that the antibacterial activity of nisin Z against *Listeria monocytogenes* (ATCC 7644) and *Bacillus subtilis* (ATCC 33712) was reported to be synergistically increased when it was tested with thymol at sub inhibitory concentration. Recently, the synergy of bacteriocins has been described by many scientist (Sivarooban *et al.*, 2008; Martínez *et al.*, 2008; O’Flaherty *et al.*, 2005; Dajcs *et al.*, 2000; Becker *et al.*, 2008). The molecular basis of synergistic action of both was postulated by Nascimento *et
The increased in activity of Lys H5, might be due to permiabilization of cytoplasmic membrane created by nisin of reagent facilitating entry of bacteriocin (Nascimento et al., 2008).

### 1.4.6 Biosynthesis of bacteriocins

Most bacteriocins are synthesized by ribosomes as biologically inactive precursor called prepropeptides (Figure 1.5). This inactive bacteriocin has a C-terminal propeptidic domain (probacteriocin) and a N-terminal extension (Jack et al., 1995, Nes et al., 1996) and this N terminal part gets recognized due to presence of glycine residues (Havarstein, et al., 1994, Cintas et al., 1997; McCormick et al., 1996 , Worobo, et al.,1995), cleaved off by specific mechanism and transported to the exterior of the cell through ABC transporters (Van Belkum et al., 1997 Havarstein, et al., 1995). In the biosynthesis of Gram-negative bacteriocins a SOS system is operated which comprises of an inducible gene rec A. This gene induced in stress condition and repressive lex A gene binds to the bacteriocin promoter and prevents its transcription (Cascales et al., 2007; Nakayama et al., 2000; Sano et al., 1993). Matsui et al. (1993) described a p box in the promoter region instead of the SOS system. RecA slices the PtrR transcriptional inhibitory protein, which becomes inactivated resulting in expression of the transcriptional activator ptrN gene. PtrN binds to the P-box and induces the expression of the pyocin genes (Matsui et al., 1993, Michel-Briand and Baysse, 2002). Alternatively, Gram positive bacteria synthesize its bacteriocin via specific biosynthetic pathway. For example, cell density dependent regulation of nisin biosynthesis (Eijsink et al., 2002). However, mechanistic regulation of their biosynthesis is still under investigation.
1.4.7 Detection of bacteriocin activity

The journey for the discovery of a novel bacteriocin like therapeutic entities is a multi step process. It proceeds from the isolation and selection of bacteriocin producing isolates from a heterogeneous population growing in a particular natural habitat. The most commonly used methods for detection of bacteriocin activity are, dual culturing method, spot lane methods, agar well diffusion method and microtitre plate method. Among these, agar well diffusion method is most popularly used for detection of bacteriocin (Jack et al., 1995). The diffusion of bacteriocins is the result of developmental gradient due to gradual exhaustion of the bacteriocin diffusion \( (x = r - rd) \) (Hoover and Steenson, 1993) (Figure 1.6). However, the constant diffusion of bacteriocin depends on various factors like molecular weight of bacteriocin, its ionic charge, the composition of the gel, temperature and solvent viscosity of the gel.
1.4.7.1 Bacteriocin detection by agar spot method

In this test, indicator strain is spread on the solid agar medium and the organism to be tested for bacteriocin activity is spotted on the surface of indicator inoculated solid agar medium and produced zone of inhibition around the spotted colonies are analyzed (Callewaert et al., 1999; Dykes et al., 2003). The bacteriocin activity of lactococcin 972 produced by Lactococcus lactis IPAL972 has been determined by spot lane assay (Martinez et al., 1999; Martinez et al., 2000). Similarly, Ohmomo et al. (1998) determined the bacteriocin-like activity of thermophilic lactic acid bacteria from the agar spot test.

1.4.7.2 Bacteriocin detection by Co culture method

In this method bacteriocin producer and bacterial indicator, both are grown in a broth and growth inhibition was determined by counting the colonies of the indicator strain. The inhibitory effect of Lactococcus lactis
subsp. lactis LL102 grown in M17 broth has been detected by this technique (Akcelik, 1999).

1.4.7.3 Bacteriocin detection by Disc diffusion method

The disc diffusion assay has also been used for detection of bacteriocin activity. In this method, a pre sterilized filter disc (8mm) containing nearly 30µl of produced, neutralized, catalase treated cell free supernatant, is placed onto freshly seeded indicator medium. After incubation, the zones of inhibition are analyzed. (Ryser and Richard, 1992).

1.4.7.4 Bacteriocin detection by Agar well diffusion method

This method is most popularly used for detection of bacteriocin activity. In this method, a well was created in to freshly indicator strain agar plate. The well was filled with either cell suspension or cell free supernatant of bacteriocin producer. Kelly et al. (1996) have been utilized this technique for determination of inhibitory activity of Lactobacillus plantarum against Listeria monocytogenes.

1.4.7.5 Bacteriocin detection by the micro plate method

A new screening method using micro plate well, for determination of bacteriocin activity from lactic acid bacteria has been described (Yashushi et al., 1997). This method was found simple, fast or time saving and economical over the conventional agar well diffusion method. In addition, a large number of bacterial samples are processed with this method (Yashushi et al., 1997).

1.4.7.6 Bacteriocin detection by Matrix-Assisted Laser Desorption/ Ionization Time-of-Flight Mass Spectrometry

The detection of bacteriocin from cell free supernatant was described by Natisha et al. (1999). This method is easy and fast, and rapid detection of nisin, pediocin, brochocin A and B, and enterocin A and B from culture supernatants has been achieved. A 0.5µl sample of bacteriocin containing cell free supernatant was adsorbed on the membrane, and then air dried and
washed with water. The maximum impurities from cell free supernatant has been removed by a 30s water wash while 60s washed degraded the bacteriocin. The adsorbed sample was dissolved in sinapinic acid in 0.1% TFA–acetonitrile (2:1) and subjected for detection of bacteriocin by MALDI-TOF-MS. The obtained resulting spectra and sequence indicated the respective bacteriocin (Natisha et al., 1999).

1.4.8 Production of bacteriocin

1.4.8.1 Production media for bacteriocin

Bacteriocin productions from many Bacilli have been reported in MRS broth, LB broth, tryptic soy broth and some food grade media. Most of the bacteriocin production has been recovered from batch culture either using free cells or immobilized cells. It was also point out that optimal growth and maximum bacteriocin production have been observed with some complex media like MRS and Elliker broths (De Vuyst , 1995).

1.4.8.2 Role of Producer strains and fermentation parameters

The production of bacteriocin is depends upon phenotypic characters like cultural features of the isolate producing bacteriocin. Thus, A newly isolate can be identified taxonomically on the basis of physico-biochemical and molecular characters. The FAME and 16S rRNA amplified gene sequence analysis are commonly used to identified a strain. Bacteriocin production depends upon the expression level of bacteriocin gene as well as enzyme activities required for conversion of inactive bacteriocin in active bacteriocin. Induction of bacteriocin expression gene depends upon the availability of some nutritional or signaling molecules. A ceiling for bacteriocin production has been observed by Kim et al. (2010). He stated that ceiling and threshold depend is affected by the presence of nutritional components and nisin inhibition. Moreover, bacterial strains are very specific
toward the nutritional sources and culture conditions (Halami and Chandrashekar, 2005; Pasic et al., 2013; Simsek et al., 2009).

1.4.8.3 Production systems

It has been found from the previously described work that most of the bacteriocins have been produced by batch culture using complex media. However, fed batch culture and continuous culture have also been reported for the production of some bacteriocin. The increased yield of pidiocin and cell mass of *P. acidilactici* NRRLB-5627 have been observed in re-alkalized fed-batch fermentation as compare to batch fermentation (Guerra and pastrana, 2001). However, packed-bed bioreactor has been used for continuous production of pediocin PO2 using immobilized *P. acidilactici* PO2. The continuous process has also been described for the production of other bacteriocin (Hoover et al., 1988; Lozano et al., 1992). The optimum yield of this anti listerial bacteriocin has been recorded with a dilution rates of at least 1.19 day⁻¹ and at the controlled pH 4.5. Optimal nisin activity in an immobilized cells system was observed in M17 media, at 31°C with 0.2–0.3/h dilution rate (Liu et al., 2005).

1.4.9 Strategies for Recovery and Purification of Bacteriocins

A purified form of bacteriocin is beneficial not only as therapeutic agents, but also to understand the mechanism of action and structural characterization. The basic components involved in purification are, production, concentration of bacteriocin, solvent extraction or salting out by ammonium sulfate and chromatographic methods of purification (Table 1.2). The purified protein is then subjected to mass and structural analysis. A good protocol of bacteriocin purification offers nearly 90% purity and more than 50% recovery (Schobitz et al., 2006).

Bacteriocin production was achieved by inoculation of mother culture of producer strain (1-5%), to a sterilized production medium and incubated
the culture at appropriate conditions required by the producer. After the completion of production duration, only the cells were removed by centrifugation (10000,-120000 rpm for 10 minutes) and bacteriocin containing cell free supernatant was recovered. The cell free supernatant was concentrated by using membrane filter of a particular molecular cut off (Garsa et al., 2014).

After concentration or reducing the volume, the bacteriocin recovery from concentration of CFS has been described by ammonium sulphate, or solvent precipitation (Yang et al., 1992). The bacteriocin from CFS was recovered by slow addition, till the complete dissolution of ammonium sulphate at 4°C. The bacteriocin recovered by precipitation are consequently liquefied in deionized water or in a weak buffer like Tris-NaCl buffer of pH (7.2-7.4) Although salting out of bacteriocins are used mainly to trim down the working volume. However, they do not provide a high intensity of purification (Guyonnet et al., 2000). Thus, subsequent steps of isoelectric focusing or chromatographic techniques, like ion exchange, gel permeation, hydrophobic interaction, HPLC and reverse-phase liquid chromatography are commonly employed to purify a bacteriocin (Garsa et al., 2014).

Hydrophobic amino acids in structure of bacteriocin are generally directed away from molecular surfaces. However, hydrophobic groups that are adequately exposed over the surface of protein molecule, provide stable conformations which are relatively available for interaction with hydrophobic ligands on the support. Compared to reverse phase chromatography, the biological activity of bacteriocin can be preserved by maintaining the low density of ligand in matrix because it offers mild elution conditions. The protein salting out by ammonium sulfate or eluted in high salt concentrations are excellently purified by HIC.
In HIC, a high ionic strength buffer (3 M NaCl/2 M Ammonium sulfate) is used to retain the protein in column. To precipitate bacteriocins, the solubility of the desirable protein under the appropriate conditions need to be checked. However, gradient elution or stepwise was used to elute the protein.

In gel filtration (GF) chromatography the high molecular weight proteins are excluded first from the GF column under the influence of steric effect of matrix which limits the degrees of access of molecules. While in ion-exchange chromatography (or ion chromatography), the protein molecules are separated according to their nature of the charge. The ionic functional groups (R-X) of stationary phase attract analyte ions of opposite charge which are retained in the column through ionic or columbic interaction. Thus, based upon nature of charge, ion exchange chromatography is further sub grouped into cation exchange chromatography and anion exchange chromatography.

Usually, but not always, the yields obtained by these methods are less than 20% (Jack et al., 1995). This might be explained by the involvement of many steps in the protocol, leading to tedious, time consuming and subsequently low yields process. The bacteriocin, optimally produced with complex medium containing mixture of nutrient components However, purification might be interfering by high content of protein (Mackay et al., 1997). A highly purified (7,834-fold purification) but low yield (3.9%) bacteriocin of *P. pentosaceus* FBB61 has been recovered from CFS by dialysis and solvent extraction. Similarly, a high yield of *pediocin L50* bacteriocin from *P. acidilactici*, has also been recovered by ammonium sulfate precipitation followed by hydrophobic interaction chromatography and reverse-phase HPLC (Cintas et al., 2001).

The bacteriocin from *E. faecium* MMT21 and *pediocin PA-1* produced by *P. acidilactici* have been purified by using three major steps of purification like (1) ammoniumsulphate precipitation, (2) chloroform/methanol
extraction/precipitation, and (3) chromatographic methods like cation-exchange/hydrophobic interaction/reverse-phase high-pressure liquid chromatography (Ghrairi et al., 2008; Fimland et al., 1996). After the recovery of purified bacteriocin, it was subjected to purity check by electrophoresis and characterized by mass spectrometry. Most of the purified bacteriocins have been analyzed by SDS-PAGE. However, in most cases the purification processes are time-consuming and accompanied by some difficulties. Thus, to avoid these, detection characterization of bacteriocin at an early stage of production has also been described by mass spectrometry (Zendo et al., 2008). MS analysis offers us not only screening of bacteriocins but also to recognize novel bacteriocins by accurate mass determination. Electron spray ionization mass spectrometry (ESI-MS) is another ionization method of bacteriocin characterization, in which multiple charge species of bacteriocin are produced and it often suitable for liquid chromatography/mass spectrometry (LC/MS) (Zendo et al., 2008).
### Table 1.2: Purification of different bacteriocin using different approaches of purification

<table>
<thead>
<tr>
<th>Bacteriocin</th>
<th>Producing bacteria</th>
<th>Method of concentration</th>
<th>Purification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enterocin A</td>
<td>Enterococcus faecium CTC 942</td>
<td>Amm. Sulphate</td>
<td>CEX, GF, RP-HPLC</td>
</tr>
<tr>
<td>Enterocin B</td>
<td>Enterococcus faecium t136</td>
<td>XAD 16</td>
<td>CEX, GF, RP-HPLC</td>
</tr>
<tr>
<td>Enterocin 96</td>
<td>Enterococcus faecalis WHE96</td>
<td>Ultra filtration</td>
<td>CEX, RP-HPLC</td>
</tr>
<tr>
<td>Enterocin MR 99</td>
<td>Enterococcus faecalis MR99</td>
<td>Amm. Sulphate</td>
<td>HIC</td>
</tr>
<tr>
<td>Enterocin AS-48 RJ</td>
<td>Enterococcus faecalis RJ 99</td>
<td>Amm. Sulphate</td>
<td>Dialysis, GF</td>
</tr>
<tr>
<td>Enterolysin A</td>
<td>Enterococcus faecalis LMG2333</td>
<td>Amm. Sulphate</td>
<td>CEX, GF, RP-HPLC</td>
</tr>
<tr>
<td>Enterocin SE K4</td>
<td>Enterococcus faecalis SE-K4</td>
<td>Cell ad-desorption</td>
<td>Solid phase ext, RP, HPLC</td>
</tr>
<tr>
<td>Enterocin AS-48</td>
<td>Enterococcus faecalis sp. liquafaciens</td>
<td>Amm. Sulphate</td>
<td>CEX, GF, RP-HPLC</td>
</tr>
<tr>
<td>Bacteriocin E 50-52</td>
<td>Enterococcus faecium</td>
<td>Amm. Sulphate</td>
<td>CEX, RP-HPLC</td>
</tr>
<tr>
<td>Bacteriocin T 8</td>
<td>Enterococcus faecium T 8</td>
<td>Amm. Sulphate</td>
<td>CEX, RP-HPLC</td>
</tr>
<tr>
<td>Enterocin F 58</td>
<td>Enterococcus faecium F 58</td>
<td>Amm. Sulphate</td>
<td>CEX, GF, RP-HPLC</td>
</tr>
<tr>
<td>Enterocin Q</td>
<td>Enterococcus faecium L50</td>
<td>XAD -16</td>
<td>CEX, GF, RP-HPLC</td>
</tr>
<tr>
<td>Enterocin L 50 B</td>
<td>Enterococcus faecium L50</td>
<td>XAD -16</td>
<td>CEX, GF, RP-HPLC</td>
</tr>
<tr>
<td>Enterocin L 50 A</td>
<td>Enterococcus faecium L50</td>
<td>XAD -16</td>
<td>CEX, GF, RP-HPLC</td>
</tr>
<tr>
<td>Enterocin P</td>
<td>Enterococcus faecium P 13</td>
<td>XAD -16</td>
<td>CEX, GF, RP-HPLC</td>
</tr>
<tr>
<td>Enterocin CRL 35</td>
<td>Enterococcus faecium CRL 35</td>
<td>XAD -16</td>
<td>CEX, GF, RP-HPLC</td>
</tr>
<tr>
<td>Plantaricin A</td>
<td>Lactobacillus plantarum C11</td>
<td>Amm. Sulphate</td>
<td>CEX, GF, RP-HPLC</td>
</tr>
<tr>
<td>Plantaricin S</td>
<td>Lactobacillus plantarum LPC 010</td>
<td>Amm. Sulphate</td>
<td>CEX, GF, RP-HPLC</td>
</tr>
<tr>
<td>Plantaricin</td>
<td>Lactobacillus plantarum</td>
<td>Amm. Sulphate</td>
<td>CEX, GF, RP-HPLC</td>
</tr>
<tr>
<td>Plantaricin 1.25</td>
<td>Lactobacillus plantarum 1.25α</td>
<td>Amm. Sulphate</td>
<td>CEX, GF, RP-HPLC</td>
</tr>
<tr>
<td>Plantaricin</td>
<td>Lactobacillus plantarum C 19</td>
<td>pH mediated adsorption desorption</td>
<td>RP-HPLC</td>
</tr>
<tr>
<td>-------------</td>
<td>-------------------------------</td>
<td>----------------------------------</td>
<td>--------</td>
</tr>
<tr>
<td>Plantaricin TMW 1.25</td>
<td>Lactobacillus plantarum 1.25β</td>
<td>Amm. Sulphate</td>
<td>CEX, GF, RP-HPLC</td>
</tr>
<tr>
<td>Plantaricin C 8 α, C β,</td>
<td>Lactobacillus plantarum NC 8</td>
<td>Amm. Sulphate</td>
<td>CEX, GF, RP-HPLC</td>
</tr>
<tr>
<td>Plantaricin 423</td>
<td>Lactobacillus plantarum 423</td>
<td>Amm. Sulphate</td>
<td>Dialysis, Chloroform methanol extraction, CEX,</td>
</tr>
<tr>
<td>Plantaricin ASM 1</td>
<td>Lactobacillus plantarum A 1</td>
<td>Amm. Sulphate</td>
<td>CEX, GF, RP-HPLC</td>
</tr>
<tr>
<td>Sakacin A</td>
<td>Lactobacillus sake LB 706</td>
<td>Amm. Sulphate</td>
<td>CEX, RP-HPLC</td>
</tr>
<tr>
<td>Sakacin S</td>
<td>Lactobacillus sake L 45</td>
<td>Amm. Sulphate</td>
<td>AEX CEX, GF, RP-HPLC</td>
</tr>
<tr>
<td>Sakacin P</td>
<td>Lactobacillus sake LTH 673</td>
<td>Amm. Sulphate</td>
<td>CEX, HIC, RP-HPLC</td>
</tr>
<tr>
<td>Sakacin C 2</td>
<td>Lactobacillus sake C 2</td>
<td>Cold ethanol PPT</td>
<td>GF</td>
</tr>
<tr>
<td>Acidocin J 1132α</td>
<td>Lactobacillus acidophilus JCM 1132</td>
<td>Amm. Sulphate</td>
<td>CEX, GF, RP-HPLC</td>
</tr>
<tr>
<td>Acidocin J 1132β</td>
<td>Lactobacillus acidophilus JCM 1132</td>
<td>Amm. Sulphate</td>
<td>CEX, GF, RP-HPLC</td>
</tr>
<tr>
<td>Acidocin D 20079</td>
<td>Lactobacillus acidophilus DSM 20079</td>
<td>Amm. Sulphate</td>
<td>Dialysis, CEX, HIC</td>
</tr>
<tr>
<td>Curvacin A</td>
<td>Lactobacillus curvatus LTH 1174</td>
<td>Amm. Sulphate</td>
<td>CEX, HIC, RP-HPLC</td>
</tr>
<tr>
<td>Curvacin 28 a, 28b, 28c</td>
<td>Lactobacillus curvatus CWBI-B 28</td>
<td>Amm. Sulphate</td>
<td>HIC, RP-HPLC</td>
</tr>
<tr>
<td>Pentocin 31-1</td>
<td>Lactobacillus pentosus 31-1</td>
<td>Amm. Sulphate</td>
<td>Dialysis, SP sepharose Fast Flow</td>
</tr>
</tbody>
</table>
1.4.10 Antimicrobial spectrum of bacteriocins

Bacteriocin activities against various Gram-negative and Gram-positive bacteria have been extensively described (Figure 1.7). Due to the presence of a permeability barrier in the cell membrane of Gram-negative bacteria they are found hard to treat not only by antibiotics but also with bacteriocins. Bacteriocin like nisin, produced by L. lactis subsp. lactis, and pediocin, produced by Pedicoccus pentosaceus has been found active against Gram-negative bacteria (Moreno et al., 1999; Stevens et al., 1991). The other bacteriocin have also been described by many scientists. Plantaricin 35d, a bacteriocin secreted by Lactobacillus plantarum has been found to inhibit the growth of Aeromonas hydrophila (Messi et al., 2001), Lactobacillus pentosus also has reported to produce a bacteriocin ST151BR (Torodov and Dicks, 2004) and Thermophylin, produced by Streptococcus thermophilus have been found to inhibit the growth E. coli, Yersinia pseudotuberculosis and Yersinia enterocolitica (Lade et al., 2006). Lactobacillus species isolated from agro-based waste also have been described the inhibitory potential of L. plantarum and L. lactis against E. coli. Bacteriocins of various bacillus species have been reported to inhibit Gram-positive bacteria like Listeria monocytogenes and Salmonella typhimurium (Ivanova et al., 1998). Bacteriocins ST28MS and ST26MS, recovered from molasses isolate namely L. plantarum has been (Torodov and Dicks, 2005) inhibited not only Escherichia coli and Acinetobacter baumanii but also some Gram positive bacteria.

1.4.11 Potential application of bacteriocins

Being a potential inhibitor of various pathogens (Figure 1.7), specific action, non resistance and nontoxic nature of bacteriocins have increased the interest of numerous scientists to lead the work in the field of bacteriocins medicines (Christopher and John, 2012). Many bacteriocins have been found
active against MDR pathogens because N-caffeoyl phenalkylamide like derivatives inhibits the bacterial efflux mechanism and nisin like bacteriocin has been reported to trigger Lys44 activity, required for ion-non specific dissipation of the proton motive force (Michalet et al., 2007; Nascimento et al., 2008). The antimicrobial potentialities of various bacteriocins against various microbial pathogens have been reported. The potential application in the field of human health, food preservation, as agricultural bio-control and marine applications are represented in the figure 1.7

![Diagram of potential fields of bacteriocins](image.png)

**Figure 1.7**: Potential field of bacteriocin application

### 1.4.11.1 Hospital acquired infections

Inhibitory potentials of many bacteriocins have been evaluated *in vitro* as well as *in vivo*, in hospital acquired infections. Nisin and lacticin 3147 significantly inhibited various pathogens in the liver, spleen and kidney. *S. aureus*, *enterococci* and *pneumococci strains* showed resistance to six or more antibiotics and difficult to treat. Therefore, some bacteriocins have been
described to treat MDR pathogens. Bacteriocin like lacticin 3147 and nisin A have been found effective against methicillin- resistance S. aureus (MRSA) and Vancomycin resistant enteroccci (VRE) (Piper et al., 2009). In vivo, the inhibitory potential of lacticin 3147 has also been evaluated against luminescent S. aureus using BALB/c mice infected with S. aureus Xen 29, treated subcutaneously with the lantibiotic (50.85 mg/kg of Ltna and 43.8 mg/kg of Ltnb) (Piper et al., 2012). In the, in vivo investigation of this study (Piper et al., 2012), decreased nisin activity was observed, which might be due to proteolytic degradation and/or nonspecific binding of bacteriocin (van Staden, et al., 2011; van Staden., 2012).

Haloduracin, (Bacillus halodurans C-125) and nisin, bacteriocins found active against Vancomycin resistance pathogens (Oman, 2009) and also significantly effective against Gram-negative Acinetobacter baumannii and Proteus sp while A. baumannii, Citrobacter freundii, Escherichia coli, Klebsiella pneumoniae, and Proteus spp. were sensitive to B602 (Svetoch et al., 2009). Aunpad and Bangchang (2007), isolated a Bacillus pumilus from Thailand, which was found to produce a bacteriocin that significantly showed Anti-MRSA and Anti-VRE activity. A broad spectrum, patented bacteriocin Planosporicin (MIC 2-16 µg/ml) from Planomonospora Sp. DSM14920 was found effective against S. aureus, (4 µg/ml), Streptococcus pneumoniae and (.25 µg/ml ) Streptococcus pyogenes (MIC and E50 0.5 µg/ml; 3.75 mg/kg). A Gram-positive bactericidal bacteriocin microbisporicin and Mutacin B-Ny266, an antibiotic produced by Streptococcus mutans Ny266 has also been investigated against infectious pathogens (Jabes et al., 2011; Mota-Meira et al., 2005).

1.4.11.2 Bacteriocin in respiratory tract infections

Microbes like H. influenza, M. catarrhalis, S. aureus Enterobacteriaceae and P aeruginosa. S. pyogenes, Neisseria meningitidis,
Pasteurella multocida, H. Influenza and Mycobacterium tuberculosis are major pathogens for the morbidity of respiratory tract infection (RTI) like pneumonia, otitis, rhinitis and tuberculosis worldwide. The bacteriocins against these infection has been described (Mandell et al., 2007; Pascual et al., 2008; Ghibrial et al., 2009; Knoetze et al., 2008; Kruszewska et al., 2004; De Kwaadsteniet et al., 2009).

1.4.11.3 Anti tuberculosis bacteriocin

Anti tuberculosis activity of bacteriocin E50-52 (Svetoch et al., 2008) has been significantly tested in mice C57BL/6JCit (B6) against Mycobacterium tuberculosis strain H37Rv using a standard antituberculosis drug rifampacin but observed less potent as rifampacin. In other antituberculosis studies, lacticin 3147 and nisin have been evaluated by Carroll et al. (2010). A safe commercialized aerosol of bacteriocin lantibiotic duramycin has been successfully marketed by the Orphan Pharmaceuticals AG for the treatment of cystic fibrosis (Steiner et al., 2008). The bactericidal nature of bacteriocins (Bcn) in tuberculosis also has been reported for future use (Sosunov et al., 2007).

1.4.11.4 Bacteriocin in skin diseases

Large human populations have suffered from skin and soft skin infection caused by S. aureus, Acne vulgaris, Propionibacterium acnes, S. epidermidis, Bacillus cereus, Bacillus subtilis, L. monocytogenes. Nisin and some other bacteriocin have been found effective to treat these skin diseases (De Kwaadsteniet et al., 2010; Bowe et al., 2006; Kang et al., 2009; Manosroi et al., 2010). A bacteriocin hiracin JM79 from Enterococcus hirae DCH5 and lactocyclicin Q from Lactococcus subspp. QU12 have been successfully evaluated in vitro against Staphylococcal enterococci, Lactobacilli, and L. monocytogenes (Izquierdo et al., 2009; Sa´nchez et al., 2007; Sawa et al., 2009) B. subtilis JM4 produces a bacteriocin Subpeptin JM4B found very
active against *S. aureus*, *P. aeruginosa*, *Salmonella sp.*, and *E. faecalis* (Ghobrial *et al*., 2009; Wu *et al*., 2005).

**1.4.11.5 Bacteriocin in dental infections**

*Porphyromonas gingivalis*, *Prevotella intermedia*, and *Actinobacillus Actinomycetes mcomitans* are regarded as the principal periodontal pathogens. Many bacteriocins like subtilosin A against *Porphyromonas gingivalis* (MIC = 3.125–6.25 µg/ml) (Shelburne *et al*., 2007) lacticin 3147, BLIS K12 and bacteriocin of 114-kDa from *Lactobacillus paracasei* strain HL32 against *Porphyromonas gingivalis* have been recorded to inhibit the growth of major oral pathogens. (Hammami *et al*., 2011; Howell *et al*., 1993; Galvin *et al*., 1999; Tagg, 2004).

**1.4.11.6 Bacteriocin as intestinal protractor**

Recently, many bacteriocin and lactic acid bacteria have been defined as intestinal protractor or replenishes of natural microflora, damaging during a course of chemotherapy (Lazzarini *et al*., 2008). Lacticin 3147 a bacteriocin potentially inhibits to *C. difficile*. A narrow spectrum compatable to thuricin CD from *Bacillus thuringiensis* DPC 6431 and *Ruminococcus gnatus* E1 (Crost *et al*., 2011), significantly found inhibitory to a number of clinical *Clostridium sp.* like *C. difficile*, *Clostridium histolyticum* NCIMB 503, *Clostridium indolis* NCIMB 9731, *Clostridium lituseburense* NCIMB 10637, *C. perfringens* LMG 10468, *C. perfringens* LMG 11264, and *Clostridium tyrobutyricum* NCIMB 8243.

Recently Miyauchi *et al*. (2013) demonstrated the mechanism of protection of trans epithelial barrier function in a bacteriocin producing *Lactobacillus salivarius*. Miyauchi *et al*. (2013) observations concluded that *L. salivarius* strains are widely divergent in their capacity for barrier protection, and this is underpinned by differences in the activation of intracellular signaling pathways. Furthermore, bacteriocin production seems
to have been an attenuating influence on *Lactobacillus*-mediated barrier protection (Miyauchi *et al*., 2013).

1.4.11.7 Bacteriocin in vaginosis

Millions of women at reproductive age commonly suffered from infectious pathogens like *Gardnerella vaginalis*, *Mycoplasma hominis*, *Prevotella Bolivia* and *Mobiluncus curtisii*, Moreover, conventionally used antibiotics like clindamycin and metronidazole to kill beneficial flora of the vagina. Bacteriocin lactocin 160 against *Gardnerella vaginalis* (Dover *et al*., 2007) and Subtilin A, from *Bacillus amyloliquefaciens* (Sutyak *et al*., 2008) have been reported. Kaewnopparat *et al*. (2013), has reported that *Lactobacillus fermentum* SK5 inhibited gastrointestinal pathogenic *Escherichia coli* and vaginal pathogenic *Gardnerella vaginalis* by producing bacteriocin like substances of molecular weight ≥ 10 kb. He evaluated pathogenic inhibition and film formation characteristics like auto aggregation. High surface hydrophobicity on HeLa, HT-29 and Caco-2 cells concluded that *Lactobacillus fermentum* SK5 can be used to colonies or film formation to inhibit the bacterial vaginosis (Kaewnopparat *et al*., 2013).

1.4.11.8 Bacteriocin as contraceptives

Besides antimicrobial activity, spermicidal activity of bacteriocin has received much attention (Reddy *et al*., 2004). Although spermicidal activity of nisin has been described, but due to its toxic effect on beneficial vaginal flora, it did not let for commercialization. Subtolin and lacticin 3147 bacteriocin from *Bacillus subtilis* have been evaluated safe contraceptive in animal as well as man without affecting beneficial micro flora. Subtilosin, therefore, appears to have great potential for use as a contraceptive (Sutyak *et al*., 2008). Fermentation HV6b, *Lactobacillus fermentum* HV6b (MTCC 10770) showed growth inhibition of a wide range of opportunistic pathogens of humans like Bacteroides, *Gardnerella vaginalis*, *Mobiluncus*, *Gardnerella vaginalis*, *Mycoplasma hominis*, *Prevotella Bolivia* and *Mobiluncus curtisii*,
Staphylococci, and Streptococci, associated with bacterial vaginosis in humans. He has also conducted spermicidal activity on semen of two healthy volunteers and concluded that fermenticin showed an impressive sperm immobilization and spermicidal activity which made it an attractive proposition for formulating antibacterial vaginosis and contraceptive products (Kaur et al., 2013).

1.4.11.9 **Antiviral bacteriocin**

The antiviral potential of bacteriocin and cell free supernatant of many bacteriocin producing bacteria against murine norovirus S99 (MNV), influenza A virus A/WSN/33 (H1N1), Newcastle disease virus Montana (NDV) and feline herpes virus KS 285 (FHV) have been described. Lange-Starke et al., 2013 has tested CFS of *Lb. curvatus* strain in the ration of 1:10 against the viruses MNV and H1N1. After incubation at 24 ºC for three days, he observed higher virus reduction of MNV by 1.25 log units with reference to the control. Subtilosin, a bacteriocin from *Bacillus amyloliquefaciens* has been tested against Herpes simplex virus type 1 (HSV-1). The safety and efficacy of a subtilosin-based nanofiber formulation have also been evaluated (Torres et al., 2013). This bacteriocin was found active even at non-virucidal concentrations against wild type HSV-1 while against acyclovir-resistant mutants, were active in a dose-dependent manner and did not show an inhibitory effect on virus multiplication prior to protein synthesis. A Poly vinyl alcohol (PVOH) -based nanofiber has formulated containing subtilin 2.5mg/ g fiber and has been described safe in human epidermal tissue.

1.4.11.10 **Bacteriocin in veterinary**

Dairy animals are generally suffered from mastitis, a bacterial intra mammary infection caused by *S. aureus, S. uberis,* and *Streptococcus dysgalactiae* (Varella et al., 2007). The strong inhibitory activity of many of bacteriocins against mastitis pathogens has spurred interest in their potential
application to the udder infections and commercialized. Nisin based udder
disinfectant as Wipe Out, Dairy Wipes (Immucell, Portland, ME, USA) is one
of the bacteriocin based products. Other commercial product, Mast Out
(Immucell, Portland, ME, USA) has been recently presented for FDA
approval. Lacticin 3147, an another interesting, highly inhibitory, lantibiotic
produced by L. lactis DPC3147 has been tested as a dry cow therapy in teat
seal formulations against many mastitis-causing pathogens (Crispie et al.,
2004; Crispie et al., 2005).

In recent trials, Klostermann et al. (2010) observed that 10-min
treatment with teat dip, containing lacticin 3147 reduced counts of S. aureus
(80%), S. dysgalactiae (97%), and S. uberis (90 %). A partially purified
lantibiotic lacticin NK34 was evaluated in mice against infection by
Staphylococcus species isolated from bovine mastitis. He described that two
strains, Staphylococcus aureus 69 and S. simulans 55 showed the greatest
susceptibility to lacticin NK34 by the spot-on-lawn assay. The minimal lethal
doses (MLD) for S. aureus 69 were measured at 1.53 × 10^9 cfu/mouse,
whereas the S. simulans 55 MLD was 3.59 × 10^9 cfu/mouse. Moreover, a
80% survival rate of S. aureus 69 MLD or S. simulans 55 MLD infected
mice were observed with the treatment of lacticin NK34, as compared with
control mice treated with distilled water. These data, as analyzed by Kim et
al., (2010) suggested that lacticin NK34 might be useful in the control of
bovine mastitis and systemic bacterial infection. A broad spectrum
peptides/bacteriocins geobacillus I and geobacillus II was characterized and
evaluated against Streptococcus dysgalatiae subsp dysgalactiae (Garg et al.,
2012) and other pathogens (Rea et al., 2010)

The other potential antimastitis bacteriocins, such as nisin U (Wirawan,
et al., 2006), uberolysin (Wirawan et al., 2007), and bacteriocin ST91KM
(Pieterse et al., 2010) Pep5, epidermin, epilancin K7, epicidin 280, aureocins
A70, A53, and 215FN) against *S. aureus* and *S. agalactiae* involved in bovine mastitis. Bacteriocins (morricin 269, kurstacin 287, kenyacin 404, entomocin 420, and tolworthcin 524) have been recently reported (Sandiford and Upton, 2012; Barboza-Corona *et al.*, 2009).

### 1.4.11.11 Bacteriocin against food pathogens

*Listeria monocytogenes* is a food born opportunistic pathogen causes listeriosis. *Carnobacterium maltaromaticum* UAL307 producing bacteriocins like piscicolin 126, carnobacteriocin BM1, and carnocyclin A, have been reported active against many Gram-positive bacteria pathogens, including numerous *Listeria* Sp (Salvucci *et al.*, 2012; Zuckerman, and Ben Avraham 2002; Martin-Visscher *et al.*, 2008). A class IIb bacteriocin Abp-118 from *L. salivarius* strain UCC118 has been evaluated in - vitro/in- vivo against *L. monocytogenes* and *L. salivarius* UCC118 by Corr *et al.*, (2007). Furthermore, *E. coli* H22 has been reported to produce several bacteriocins like microcin C7 and colicins 1b and E1, to inhibit several pathogenic or potentially pathogenic enterobacteria like *Enterobacter agglomerans*, *E. coli*, *K. pneumoniae*, *Morganella morganii*, *Salmonella enterica*, *S. flexeneri*, *S. flexneri* and *Yersinia enterocolitica* (Cursino *et al.*, 2006). *Enterococcus faecalis* OSY-RM6 produces a bacteriocin Enterocin RM6, which was evaluated against Gram-positive bacteria, including *L. monocytogenes*, *Bacillus cereus*, and methicillin-resistance *Staphylococcus aureus* (MRSA).

Enterocin RM6 (final concentration in cottage cheese, 80AU/ml) caused a four log reduction in population of *L. monocytogenes* inoculated in cottage cheese within 30 min of treatment (Huang *et al.*, 2013). Recently the antimicrobial potential of *Lysinibacillus* against food borne bacterial and fungal pathogens, has been reported (Ahmad *et al.*, 2014). Beside fungal inhibitor, this isolates strongly inhibits the foodborne Gram-positive microbial pathogens such as *Staphylococcus aureus*, (22 mm ZOI), *Staphylococcus*
epidermis and Bacillus cereus (18 mm). Furthermore, Chopra et al., 2014 has reported a potential biocontrolling bacteriocin sonorensin, might be belonging to a new Sub-class bacteriocin hetrocyloantrocin which was found active against Staphylococcus aureus and L. monocytogenes.

1.4.11.12 Food preservatives applications of bacteriocin

Ever since the era of Louis Pasteur and Robert Koch, there has been scientific recognition of an essential need to prevent the growth of food spoiling microbial flora. Regardless of the fact that, many chemicals and antibiotics are used to preserve many food products but they are not the first choice of consumers, where they like food with fresh or natural preservatives. Thus, many food products got a wide acceptance and commercialized due to the level of “foods with no chemical preservatives added”. Over the last two decades, many bacteriocins, produced by Gram-positive and Gram-negative bacteria that kill or inhibit the growth of the food spoiling organism, have been identified, characterized and evaluated for food preservations. The common food spoiling bacteria are Enterococcus, Lactobacillus, Lactococcus, Leuconostoc, Pediococcus, and Streptococcus, but also inhibit many less closely related Gram-positive bacteria, such as L. monocytogenes, Staphylococcus aureus, Bacillus cereus, and Clostridium botulinum. Biopreservation of the food as bacteriocin additives are achieved by inoculating producer strains itself or its or purified/semi purified bacteriocin (Schillinger et al., 1996; Dabour et al., 2009).

L. monocytogenes is common meat pathogen grown in low availability of moisture content, even at refrigerated temp. Thus, due to easily growing capabilities, food contamination by L. monocytogenes has become a serious threat to public health worldwide. The United States government has the greatest rigid policy and set a zero tolerance level for L. Monocytogenes in ready-to eat foods (Jay., 1996; Ryser and Marth., 1999). The most potent and
tested bacteriocin nisin has been evaluated and found a significant inhibitory effect against *L. monocytogenes*, incubated for 16 days on the raw meat. Recently, food preservatives and sanitation efficiency of a cyclic peptide Enterocin AS-48 have been evaluated against *Listeria monocytogenes* and different cellular responses of *L. monocytogenes* to interacting with AS-48 in the planktonic and sessile state, (including stress response and cell metabolism) proteins have been analyzed (Caballero *et al.*, 2013).

An another broad spectrum enterocin RM6 *Enterococcus faecalis* has been evaluated against food pathogens, Gram-positive bacteria *L. monocytogenes*, *Bacillus cereus*, and methicillin-resistance *Staphylococcus aureus* (MRSA) but found potential inhibitors to *monocytogenes*( 80 AU/mL caused a 4-log reduction) Espitia *et al.*, (2013) evaluated physical-mechanical and antimicrobial properties of nanocomposite film with pidocin and ZnO nanoparticles against *Staphylococcus aureus* and *Listeria monocytogenes*. Further, the biopreservative potential of nisin incorporated film has been evaluated against *Staphylococcus aureus* and *Listeria monocytogenes* on mango slice packaging and concluded that the population of inoculating indicator was reduced from (10(7)CFU/g) to (10(5)CFU/g) (Barbosa *et al.*, 2013). The food preservative potential of newly isolated bacteriocin Gassericin A (GA), isolated from *Lactobacillus gasseri* LA39 has been recently described by Nakamura *et al.*, 2013. This bacteriocin is a circular bacteriocin which was found stable at 4°C for 3 months, 37°C for 2 months, 60°C for 5 h, and 100°C for 30 min.

**1.4.11.13 Application of bacteriocin in marine food**

The population of Japan like countries dominantly depends on seafood for the sustainability of life. It has been analyzed by FDO that there has been a nearly fourth time increased in demand for seafood since 1986. Marine fishes were suffered from various diseases like Furunculosis, vibriosis,
columnaris disease, streptococcusis, pasteurellosis, fish tuberculosis, and enteric septicemia predominantly caused by *Aeromonas*, *Vibrio*, *Cytophaga*, *Streptococcus*, *Pasteurella*, *Mycobacterium*, and *Edwardsiella* genera. These diseases contribute major economic losses of any country whose population depend on sea food.

It was proven from previously conducted research that bacteriocins produced by marine bacteria significantly have been used as probiotics and antibiotics in the seafood industry (Galvez *et al*., 2008; García *et al*., 2010; Pilet and Leroi, 2011). The first marine bacteriocin harveyicin was isolated from marine micro-organisms *Vibrio harveyi* (McCall and Sizemore, 1979). Previously conducted research proven that bacteriocin is a safe and effective peptide antibiotic to control the diseases and spoilage of sea food. Because of bearing beneficial properties (Galvez *et al*., 2008) bacteriocin became the alternative choice of food preservative as spray drying, direct incorporation in packaging, use of bacteriocinogenic strain or in combination of other preservative to retain the synergistic action at the safe level of concentration (Calo-Mata *et al*., 2007; Galvez *et al*., 2007). Brillet *et al*. (2005) evaluated the bacteriocinogenic potentialities bacteriocin producer *Carnobacterium divergens* V41 against *Listeria monocytogenes* in cold smoked salmon and concluded that spraying application of *C. divergens* did not affect the sensory qualities of the salmon produced even commercial scale (Brillet *et al*., 2005). Further, Schobitz *et al*. (1999) directly applied a BLIS from *Carnobacterium piscicola* into vacuum-packed meat and stated that it inhibited the growth of *L. monocytogenes* in the vacuum-packed meat after 14 days of storage at 4 °C.

Recently, Gebreselema *et al*. (2013) has evaluated the crude extract of an *Actinomycetes* isolated from water and sediments of Lake Tana, Ethiopia, against Gram-positive *S. aureus* and Gram negative *E.coli* and significant inhibition nearly 95% were observed (Gebreselema *et al*., 2013). Similarly,
Valli et al. (2012) isolated 21 potential *Actinomycetes* from the oceanic environment and reported that all the isolates were promising against at least one tested organism. The Synergistic action of many bacteriocins has been evaluated and found to be effective and safe with currently used sea food preservatives (Calo-Malta et al., 2007; Galvez et al., 2007; Pilet and Leroi, 2011; Nykanen, 2000). Bacteriocin impregnated in Polyethylene film and/or gel has been described by (Neetoo et al., 2008).

The preservative potential of bacteriocin in sea food has also been described by many researchers (Aasen et al., 2003; Al-Holy et al., 2004; Elotmani and Assobhei, 2004; Luders et al., 2003; Neetoo et al., 2008; Nilsson et al., 1997; Nykanen et al., 2000; Szabo and Cahill, 1999; Tahiri et al., 2009; Tsironi and Taoukis, 2010; Bushra et al., 2009).

**1.4.11.14 Diagnostic applications of bacteriocin**

$^{99m}$Tc-Duramycin naturally derived peptide antibiotics (bacteriocin) obtained from *Streptoverticillium cinnamoneus* and made up of 19 amino acid residues. It has high affinity and specificity to words phosphatidyethanolamine (PE) which have been utilized to recognize apoptotic and necrotic cells. Labeling of biomolecules like DNA, protein etc, by radioactive or fluorescent markers is one of the most commonly used methods for numerous qualitative and quantitative bio-analytical purposes in clinical medicines (Sameiro and Gonalves, 2009). The detection of pathogens and action mechanism in *L. ivanoi* CIP 12510 (A), *L. monocytogenes* CIP 82110 (B) and *L. innocua* CIP 12511 (C), with labeled nisin has been visualized by, fluorescence ratio-imaging microscopy.

Imran et al., (2013) evaluated detection and antibacterial mechanistic study of nisin, and labeled nisin against three *Literia species* namely *L. ivanoi* CIP 12510 (A), *L. monocytogenes* CIP 82110 (B) and *L. innocua* CIP 12511 (C). He used nisin Z and fluorescent marker 5-(aminoacetamido)
fluorescein (AAA-flu) coupled with carboxyl group of purified nisin Z via a HOAt/EDC coupling in 100 µl DMF. Confocal intensity detection analysis of target cells showed that labeled nisin Z was located at the cell division sites of the studied bacteria and required in nano concentration for disruption of the membrane of targeted bacteria (Imran et al., 2013). This study provides an alternative or beneficial method over the traditional bio-analytical or diagnostic methods for localization of Literia species and analysis of mechanism of action.

1.4.12 Genetic engineering and bacteriocin

Knowledge of the encoding genes, biosynthetic and secretory pathways of many bacteriocins have facilitated to engineer the desirable bacteriocin with the help of various approaches of genetic engineering. In bacteriocin engineering, scientist have analyzed the gene organization of the bacteriocin producer organism and made the required modification to improve the properties of bacteriocins as significant antimicrobial agents (Kuipers et al., 1993; Engelke et al., 1994; Siegers and Entian, 1995).

The approaches like mutagenesis, gene fusion and insertions of desirable amino acid or functional group during their chemical synthesis, have been tested to engineer the various bacteriocins. Besides increasing the stability through genetic engineering, the changes in activity spectrum of some bacteriocin have been achieved. Pyocin S-35, a narrow spectrum bacteriocin, produced by P.aeurogenosa (Smith et al., 2012). Broad spectrum activity of this has been achieved by fusion and translocation (Michel-Briand and Baysse, 2002). A species specific bacteriocin against S. aureus has also been designed through fusion technology (Qiu et al., 2003).

Microcin V active against Gram-positive, a bacteriocin from E.coli, engineered with signal peptide converted its activity against Gram-negative bacteria. Stability of bacteriocin has also been increased using fusion
technology. A chimeric construct of two genes encoding the glycinicin subunits, from *Xanthomonas campestris*, resulted in retaining similar activity as the wild type. This construct also provided increased stability not only at higher and lower pH, but also at higher temperatures. The novel bacteriocin molecule has also been designed utilizing random or site directed mutagenesis. The solubility at pH 7 of nisin Z has been made by incorporation of lysine residues. Class IIa bacteriocins like pidicin PA-1, sakacin P that have been engineered by incorporation of an additional di sulphide bridge resulted in increased activity and stability. However, protease sensitivity of some bacteriocins of this class has been reduced by the incorporation of D amino acids in class IIb bacteriocin lactococcin G. Thus, these methods may be valuable routes for stabilizing bacteriocin of discrete classes (Yan *et al.*, 2000; Oppegard *et al.*, 2010).

1.4.13 Market potential or commercialization of bacteriocins

The use of nisin as food preservatives was approved by FAO/WHO in 1969. In 1988, United States FDA approved the use of nisin as a canned food preservative to inhibit the growth of *C. botulinum*. During 2000-2003, 182 food poisoning, particularly in meat, cheese and seafood were linked to contamination by *Listeria* species. A soy-based plastic film containing nisin and lauric acid also has been commercialized to control the growth of *Listeria* spores. Thus, in 2003, Danisco’s Nisaplin significantly inhibited the viable count of pathogenic *Bacillus* and *paenbacilllus sp*. Another example, which is commercialized aerosol of lantibiotic duramycin as Moli 1901 has been successfully marketed by AOP Orphan Pharmaceuticals AG for the treatment of cystic fibrosis (Steiner *et al.*, 2008; Grasemann *et al.*, 2007). The use of bacteriocin preservatives, become part of the $22 billion global food additives market and average annual growth rate of 2.4% per annum between 2001 and 2004 has been estimated. This market is expected to grow at 2-3% per annum.
A nisin based udder disinfectants as Wipe Out, Dairy Wipes (Immucell, Portland, ME, USA) are one of the bacteriocin based product which has been commercialized. Another commercial product, Mast Out (Immucell, Portland, ME, USA) has been submitted recently for FDA approval. Royal DSM, NV and Kerry Biosciences Netherlands, Rhodia, S.A., France, Sysco Foods’ China, Schreiber Foods U.S. are the organizations that are currently involved in, to explore the acceptance of the bacteriocin based product among the consumers.

1.5 CONCLUSION

Production of protective or signaling molecules is a widespread phenomenon among bacteria. Being safe and natural origin, the demand of bacteriocin consumption among the consumer has been approved worldwide. The antimicrobial properties of bacteriocin have been described against Gram-positive, Gram-negative and fungal pathogens. Therefore, the inhibitory potential of bacteriocins has been established in the field of human health care, particularly to control the infectious diseases, animal health care, food preservation and in the agriculture. Furthermore, a serious threat of antibacterial resistance has been decreased by nisin like bacteriocin (Hurst, 1981). The inhibitory activities of many bacteriocins have been conducted in-vitro as well as in-vivo and some bacteriocins have been reported with high therapeutic efficacy. The therapeutic efficacy of many bacteriocins has been increased by generating engineered bacteriocins, produced by diverse approaches of genetic engineering. Further, factors like cell growth, expression level, localization, modification and regulation are important factors that should be considered to engineer a bacteriocin as safe and effective alternative drug molecule.