BACTERIOCIN AND ITS APPLICATION – A REVIEW

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ABSTRACT

Bacteriocins were first detected by Andre Gratia in 1925. They are ribosomally synthesized extracellularly released low molecular mass peptides, produced by different types of bacteria, Gram positive, Gram negative and Archaea. They can be produced spontaneously or induced by certain chemicals such as mitomycin C. They are broadly classified into four groups; class I termed Lantibiotics, class II (small heat-stable peptides), class III (large heat-labile peptides) and class IV (large complex bacteriocin). Mode of action is bacteriocidal or bacteriostatic. Bacteriocin possesses several applications that include in animal production, human health, as a bio preservative agent, animal production in medicine, in aquaculture and active against phytopathogens.

Keywords: Application, Bacteriocin, Bacteria and Peptides.

INTRODUCTION

Bacteriocins are ribosomally synthesized extracellularly released low molecular mass peptide or proteins. They are proteinous toxins that are active against other bacteria either of the same specie (narrow spectrum) or across genera (broad spectrum). They are generally recognized as "natural" compounds able to influence the safety and quality of foods (Zavala *et al.,* 2007).

Bacteriocins are antimicrobial compounds that are produced by many different bacterial specie including lactic acid bacteria (LAB) (Jennifer *et al.,* 2001). Some bacteriocin produced by LAB inhibit not only closely related species, but are also effective against food borne pathogens such as *Listeria monocytogenes* and many Gram – positive food spoilage causing microorganisms (Svetoslav, 2009).

Bacteriocin Discovery and Production

Bacteriocins were first detected in 1925 by Andre Gratia who observed that the growth of some *E.coli* strains was inhibited by the presence of an antibacterial compound, which he called colicin V, released into the medium by *E.coli* V (virulent strain). Colicin V was later characterized as a heat-stable dialyzable peptide compound and, in 1954, Pierre Frederic found its genetic determinants in a conjugation-transmissible element similar to the F factor (Fredeririq, 1954). The production of small antibiotic peptides is a common defence strategy against bacteria that is displayed not only by microorganisms but also by animals and plant. Magainins, cecropins and defensins are animal and thionins are plant (Hillman *et al.,* 2000) antimicrobial peptides. These antimicrobial vary significantly in their amino acid sequence, but most of them share features such as low molecular weight, heat stability and a cationic and hydrophobic nature. Furthermore, all of them are coded for by structural genes that are ribosomally translated into peptides.

Bacteriocin of Gram – Positive Bacteria

The production of bacteriocin in Gram positive bacteria is associated with the shift from log phase to stationary phase, the production begins during mid-log phase and progress to a maximum as it enters stationary phase (Pugsley, 1984). Gram positive bacteriocins in general require many more genes for their production than do Gram-negative bacteriocin. The nisin gene cluster include genes for the prepeptide (nis A), enzymes for modifying amino acid (nis B, Nis C) cleavage of the leader peptide (nis P), secretion (nis T), immunity (Nis I, nis FEG) and regulation of expression (nis R, nis K) (Hillman *et al.,* 2000).

The gene cluster are most often encoded on plasmids, but occasionally found on the chromosome. Several gram positive bacteriocin including nisin are located on transponos (Bromberg *et al.,* 2004).

Gram positive bacteriocin possesses a restricted killing range to killing other Gram-positive bacteria. The range can vary from relatively narrow as in the case of bacteriocin A, B, and M

(Jennifer *et al.,* 2001) to extraordinary broad, which shows activity on wide range of organisms including *Actinomyces, Clostridiuin, Corvne bacterium, Enterococcus, Gardenenella, Lactococcus, Streptococcus* (Karman and Bojana, 2003).

Production of bacteriocin and bacteriocin like substance has been reported from other Gram positive bacteria, such as members of the genus *Bacillus including B. coagulas, B. brevis B. lichniformis, B cereus, B. subtilis, B. amyloquefaciens* and other *Bacillus species* (Sharma *et al.,* 2006).

Bacteriocin of Gram – Negative Bacteria

They are principally divided into two groups; Colicins and microcins. They were operationally defined on the basis of their molecular sizes. *Colicins* are large (25 -to 80 -KDa), they are bacteriocidal proteins and their production is induced by conditions triggering the SOS system. Colicins, the first bacteriocin discovered, are characterized by their narrow antibiotic spectrum and by displaying a bacteriocidal activity mediated by interaction with specific membrane receptors. Some of them are peculiar because their synthesis is controlled by a SOS — dependent mechanisms that include suicide of the producer by cells (Pugsley, 1984). Colicin- like proteins produced by *Pseudomonas* species are called pyocins. An example is puticidin, a pyocin of 276 amino acid residues produced by *P. aeruginosa*. Microcins, on the other hand, have many structural similarities with class II bacteriocin, their molecular size is smaller than lOkDa, they are synthesized during stationary phase, and they are not under SOS control. Two peculiar members of this class are colicin V, the first bacteriocin described by Gratia in 1925, which can be considered as a microcin because of its molecular mass (6KDa), and microcin C7 a modified hepatapeptide that is considered to be the smallest antibiotic peptide described so far (Ganzalez — Pastor *et al.,*1994).

Colicin V share several features in common with class IIa bacteriocin from Gram — positive bacteria such as their sizes, double — glycine — leader — directed secretion via an ABC transporter and relative high hydrophobicity (Ganzalez — Pastor *et al.,* 1994).

Colicins and indeed all bacteriocins produced by Gram — negative bacteria, are large proteins, pore forming, ranging in size from 178 to 777 amino acids. Although colicins are representative of Gram— negative bacteriocin, there are intriguing differences found within this sub — group of the bacteriocin family. *E. coli* encodes its colicin exclusively on plasmid replicons (Pugsley and Ondega, 1987). The nuclease pyocins of *Pseudomonas aeuroginosa*, which shows sequence similarly to colicins and other yet uncharacterized bacteriocins are found exclusively on the chromosome (Klaenhamrner, 2003). Another close relative to the colicin family, the bacteriocins of *Serratia marcesers*, are found on both plasmid and chromosomes (Ferrer *et al.,* 1996).

Many bacteriocins isolated from Gram — negative bacteria appear to have been created by recombination between existing bacteriocin (Ferrer *et al.,* 1996). Such frequent recombination is facilitated by the domain structure of bacteriocin protein in colicins, the central domain

comprises of about 50% of the protein and is involved in the recognition of specific cell surface receptors. The N — terminal domain (25% of the protein) is responsible for translocation of the protein into the target cell. The reminder of the protein houses the killing domain and the immunity region, which is short sequence involved in immunity protein binding. Although the pyocins produced by *P. aeroginosa* share similar domain structure, the order of the translocation and receptor recognition domains are switched (Sano *et al.,* 1993). The conserved domain configuration of these toxins is responsible for much of the bacteriocin diversity observed in nature.

Bacteriocin of Archea Bacteria

Members of the Archaea also produce their own distinct family of bacteriocin — like antimicrobials, known as archaeocins the only characterized member is the halocin family produced by halobacteria, among these group few have been described in detail (Cerning *et al.,*1999). The first halocin discovered S8, is a short hydrophobic peptide of 36 amino acids, which is processed from a much larger pro — protein of 34KD (Price and Shand, 2000). Halocin S8 is encoded on a mega plasmid and is extremely hardy, it can be resulted boiled, subjected to organic solvent, and stored at 40°C for extended periods without losing activity. Expression is growth stage dependent. Although basal levels are present in low concentration. Sometimes basal levels are present in low concentrations during exponential growth phase, followed by an explosive nine fold increase in production during the transition to stationary phase (Shand *et al.,* 1998). The mechanisms of halocin action has been established only for Halocin H6 (aNa+H+antoporter inhibitor), and the immunity mechanisms is unknown (Torreblanca et al., 1989).

Bacteriocins produced by the Archea — Archeacins are produced as the cells enter stationary phase. When resources are limited, producing cell lyse, sensitive cells and enrich the nutrient content of the local environment long enough to reduce competition during subsequent phases of nutrient influx. The stability of halocins may help explain why there is so little species diversity in the hypersaline environments frequented by halobacteria (Shand *et al.,* 1998). As has been seen in the foregoing of bacteriocin diversity and distribution, this heterogenous family of protein based toxins, that have relatively narrow killing spectrum and often extremely hardly stable what makes them weapons of choice in the microbial world remains an intriguing question.

Bacteriocin Classification and Mode of Action

Classification of Bacteriocin

Bacteriocins are broadly classified into four groups (Jeevaratnam *et al.,* 2004). Class I termed Lantibiotics, which is further divided into class Ia and class Ib class II, also divided into IIa and other classes are III and IV.

Class I (Lantibiotics)

They belong to a family of membrane active peptides, containing the unusual ether amino acids Lanthionine and 13-methyl Lanthionine including other modified amino acids such as dehydrated serine and threonine (Jung, 1991).

Class I peptides typically possess from 19 to more than 50 amino acids. They are differentiated by their unusual amino acid such Lanthionine, methyl Lanthionine, dehydrobutyrine and dehydroalanine. The best example of bacteriocin belonging to these group is nisin produced by *Lactococcus lactics*, it consists of cationic and hydrophobic peptides that form pores in target membrane and possess a flexible structure, when compared to class Ib, that are more rigid, NL-globular peptides with no net change or a negative change (Ashok, *et al., 2014*).

Class II (small heat-stable peptide)

Contains small heat — stable, non-modified peptides. They are bioactive peptides and do not contain any modified amino acid residue such as Lanthionine (Sahl and Bierbaum, 1998). The class is sub — divided into IIa and IIb. Class IIa, include pediocin like *Listeria* active peptides with a conserved N — terminal sequence. Tyr— Gly — Asn — Gly — Val and two cysteine forming S-S bridge in the N — terminal half of the peptide. Class IIb, in these sub — class, two peptides are needed to be fully active. The primary amino acid sequences of the peptides are different. Although each is encoded by its own adjacent genes, only one immunity gene is needed (Jimenez-Diaz et al., 1995).

Class III (Large Heat Labile bacteriocin)

They are large and heat labile bacteriocin. They are large protein molecules with a large molecular weight. They include helviticin and lactacins A and B. (Ravi *et al.,* 2012).

Class IV (Large Complex Bacteriocin)

The class consists of bacteriocin that form large complex with other macro — molecules, that is, they form large complexes with other chemical moieties, carbohydrates or lipids however, no such bacteriocin has been purified (Klaenhammer, 1993).

Mode of Action

Most of the bacteriocins are bactericidal, with some exceptions (Leucocin A- UAL 187 being bacteriostatic) (Ennaher *et al.,* 2000). Due to the great variety of their chemical structures, bacteriocin affect different essential structures of the living cells, 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 (Abee, 1995).

Bacteriocins are positively charged molecules with hydrophobic patches. Electrostatic interactions with negatively charged phosphate groups on target cell membranes are thought to contribute to the initial binding with the target membrane (Chen *et al.,* 2005).

The association of hydrophobic patches of bacteriocin with the hydrophobic membrane has also been modeled using computer simulation to predict the most favourable interaction (Lavermicocca *et al.,* 2002). It is likely that the hydrophobic portion inserts into the membrane, forming pores. There is a debate over the types of pores formed by nisin, with most groups favouring the "barrel stave" or "wedge models". In the "barrel stave" model, each nisin molecule orients itself perpendicular to the membrane, forming an ion channel that spans the membrane (Onda *et al.,* 2003).

According to the "wedge" model, after a critical number of nisin insert concurrently, forming a wedge (*Galle et al., 2010*).

Bacteriocin forms pores in the membranes of target cells (Abee, 1995). It is hypothesized that the mode of action involves various step such as binding, insertion and pore formation (Montaville and Chen, 1998). Binding of the bacteriocin to the target membrane is necessary for subsequent insertion and pore formation.

Although the interaction of a reception — like factor has been implicated for some bacteriocins (Chikindas *et al,* 1993; Aly, 2007), a protein receptor does not appear to be essential for binding. It is also proposed that bacteriocin mediated transmembrane ion flow results in cytotoxic effects causing a drop in the intracellular pH and inhibiting enzymatic processes. An influx of cytotoxic sodium ions and a depletion due to fertile cycles are caused by ion gradient dissipation. Dissipation by the proton motive force and the trans-membrane potential arrest processes dependent on these gradients (Bruno and Montiville, 1993).

Bacteriocin are unstructured in an aqueous solution, but have the ability to form a helical structure when exposed to structure proting solvents, or when mixed with anionic phospholipid membranes

It is hypothesized that the highly conserved N — terminal of the class Ia bacteriocin contributes to the membrane binding. This allows the low homologous C terminals to transform from random conformations to defined secondary structures (Ashok *et al.,* 2014).

Specific amino acids play a role in the antimicrobial activity of class IIa bacteriocins. The presence of cysteine in the structure of these bacteriocins with subsequent modification of pairs of cysteine residue to form disulfide bridges affects the activity of bacteriocins (Miller *et al.,* 2013).

Aromatic amino acids are also involved with antimicrobial activity. Loss of activity when small fragments of the N — terminal or the C — terminal are removed suggests that the whole sequence of the bacteriocin is necessary for activity (Miller *et al.,* 2013; Ashok *et al.,* 2014).

The biological targets of bacterions produced by Lactic acid bacteria (LAB) are the anionic lipids of the cytoplasmic membrane, which acts as the primary receptors for initiation of pore formation (Abee, 1995; Montaville, *et al.,* 1995). Previous findings suggested a protein-receptors mediated activity (Bhunia *et al.,* 1991; Chikindas *et al.,*1993), but recent studies focusing on the effect of class IIa bacteriocins on lipid beside systems indicate that proteins receptors are not the main requirement for pore formation (Ennahar *et al.,* 2000). It has been suggested that these receptors act to determine specificity of class II bacteriocins (Venema *et al.,* 1995). Pore formation ultimately results in the leakages of inorganic phosphates and ionic imbalance (Deegan *et al.,* 2006), f3 - galactosidase, DNA and RNA material (Todorov, 2008). The initial disturbance causes dissipation of the proton motive force (PMF) which encompasses a complete or partial dissipation of either or both the pH gradient and the trans-membrane potential. For class Ia bacteriocins complete dissipation of the pH gradient occurs readily, while only a partial dissipation of the trans-membrane potential usually occurs (Ennaher *et al.,* 2000). Dissipation of the proton motive force (PMF) by class IIa bacteriocins can be considered their main action to exert lethal activity (Abee, 1995; Ennaher, *et al.,* 2000; Fimland *et al.,* 1996). ATP is depleted as much as 98.9% and active transport involved in the uptake of amino acids is blocked (Chkindas *et al.,* 1993). Leakage of pre-accumulated amino acids among various other UV-absorbing materials, has been reported for plantaricin 423 bacteriocin ST 23LD and ST194BZ produced by different strains of *L. plantarum* and this leakage may be due to the diffusion of amino acid through the pores formed by bacteriocin as visualized by atomic force microscopy (Todorov and Dicks, 2006).

In contrast to lantibiotics, class Ia bacteriocin causes no leakage of ATP. This may be due to smaller pore sizes that are formed by the action of the latter. However, ATP depletion does occur and this may result from an increased consumption of the PMF. The depletion may also be due to the efflux of inorganic phosphate that is needed to produce ATP (Ennahar *et al.,* 2000).

Three pore formation models have been described by which bacteriocins act on the cell membranes of sensitive cells, a "wedge — like", a "barrel stave"- like model or a carpet mechanisms (Kaktchman et al., 2012). Class 1 bacteriocins may function by using a wedge - like model to induce pores, whereas class II bacteriocins may form pores by either following the "barrel stave" like model or a carpet mechanisms. The carpet mechanisms are accomplished by peptides orientating parallel to the membrane, thereby interfering with the membrane structure (Kaktchman et al., 2012). Pore formation by class IIa bacteriocins using the "barrel stave — like model may be due to the peptides' putative trans-membrane helices, membrane — binding ability and water solubility (Abee, 1995; Venema *et al.,*1995). Thus so far, two mechanisms for the initial interaction between class II bacteriocins and the membrane surface have been

hypothesized, namely: (A) electrostatic binding of the bacteriocin to the membrane surface mediated by a putative receptor-type molecule bound to the membrane (Ennahar *et al.,* 2000), and (B) binding between positively charged amino acids and anionic phospholipid heads in the membrane (Ennahar *et al.,* 2000). Class II bacteriocins may rely on basically the same type of functional binding due to high structural similarities in their hydrophilic N-terminals (Ennahar *et al.,* 2000). A crucial subsequent step in the process of pore formation is the hydrophobic interaction between the amphiphilic region of the C - terminal part of the bacteriocin and the lipid acyl chains (Kaktchman *et al.,* 2012). In contrast to the N - terminal domain that plays a role in the electrostatic interaction between the bacteriocin and the membrane surface, the C-terminal is believed to be cell - specificity determining region (Fimland *et al.,* 1998).

Structural features, such as the YGNGV motif, ct-helices, disulfide bonds, and positively charged amino acids, play an important role in cell recognition and activity of class Ia bacteriocins (Ennahar *et al.,* 2000). These structural features are found within different domains spanning the bacteriocin peptide, indicating its complex nature. The 13 - sheet domain exerts antimicrobial activity, whereas the a-helix is thought to be responsible for target specificity (Fimland *et al.,* 1998). The YGNGV motif allows for correct positioning of the bacteriocin on the membrane surface as it is recognized by a putative membrane receptor due to exposure caused by a 13-turn structure (Aly, 2007). The hydrophilic/amphiphilic N-termini of the 13-sheet are another component involved in recognition, possibly due to its electrostatic membrane bacteriocin interaction. However, both the YGNGV motif and N - termini of the (3- sheet of class IIa bacteriocin) do not determine their specificity of activity phospholid layer (Ennahar, *et al.,* 2000). The central domain forms a hydrophilic or slightly amphiphilic (i-helix and is believed to play a role in destabilization of the phospholipid layers). This mediates the insertion of the bacteriocin in the cytoplasmic membrane (Ennahar *et al.,* 2000). The C- terminal hydrophobic/amphiphilic (i-helix contributes to insertion of the bacteriocin into the cytoplasmic membrane of target cells resulting in the formation of water filled pores) (Ennahar *et al.,* 2000). Furthermore, the C-terminal domain plays a role in the target — cell specificity due to its putative transmembrane helices. Another feature that has to be taken in consideration is the presence of disulphide bonds. All class IIa bacteriocins contain at least one disulphide bridge and have been shown to play a role in activity of the bacteriocin (Eijisink *et al.,* 1992). Studies investigating the spectra of activity of class Ia bacteriocins have shown that bacteriocins with two disulphide bonds displayed a greater and broader spectrum of activity in comparison with those containing only one bond.

Class llb bacterions are dependent on two distinct peptides for activity. They are responsible for dissipation of the trans-membrane potential, while only a few affect the pH gradient. These two peptide bacteriocins (class IIb) can be divided into two subgroups based on their ion-selectivity:

(A) monovalentcation conducting bacteriocins, example plantaricin EF (Park *et al.,* 1997) and (B) anion conducting bacteriocins, example plantaricin JK. Class IIb bacteriocins vary in their

modes of action, which ultimately leads to membrane permeability, pheromone activity and specific inhibition of spectrum formation (Hchard and Sahl, 2002).

Applications/Uses of Bacteriocin

Bacteriocin as a Biopreservative Agent

Consumers are very concerned of chemical preservatives and processed foods, but they accept easily lactic acid bacteria (LAB) as a natural way to preserve food and promote health (Montville and Winkowski, 1997).

Bacteriocin producing LAB has potential for the preservation of foods of plants origin, especially minimally processed vegetables such as pre-packed mixed salads and fermented ones. LAB targets food pathogens without toxic or adverse effect.

The only bacteriocins currently employed in food preservation are those produced by LAB used in the production of fermented foods (Montville and Winkowski, 1997). LAB has been used for centuries to ferment foods and enjoy the GRAS status (generally recognized as safe) status by the US Food and Drug Administration (FDA). This permits their use in foods without additional regulatory approval (Montville and Winkowski, 1997).

Nisin was the first bacteriocin to be isolated and approved for use in foods specifically to prevent the outgrowth of Clostridium botulinum spores in cheese spreads in England (Chung *et al.*,1989). By 1988, the FDA has approved its use as a biopreservative for narrow range of foods, including pasteurized egg products. Today nisin is accepted as a safe food preservative by over 50 countries, and it's the most widely used commercial bacteriocin and it remains the only bacteriocin that may be added to U.S foods.

The next wave of development of bacterriocin as food preservative is at hand. Bacteriocins have been discovered in cured meats, milk and cheese, spoiled salad dressing and soybean paste. A natural concern about using bacteriocin for the preservation of food is selection of resistant strains. Studies in LAB have shown that resistance carries a significant fitness cost with resistant strain having a slower growth rate than their sensitive ancestor. Treatment with a combination of bacteriocin, for instance nisin and a class Ia bacteriocin, would theoretically reduce the incidence of resistance (Bouttefroy and Milliee, 2000; Buchmarni *et al.*, 1998).

Bacteriocin in Animal Production

Over the years, bacteriocin research has been primarily moved by applications in food preservation and food safety. Over the last 20 years, more than 700 patents based on bacteriocins produced by LAB have been registered, and approximately 400 were linked to food preservation and to animal probiotics (http://www.freepatentsonline.com). However, many bacteriocins also show potential for biotechnological and agro - industrial applications. Some bacteriocins show desirable properties for in vivo application, such as stability to low pH and heat, simplicity for

production and extraction and little, if any, inhibitory activity towards eukaryotic cells. Therefore, bacteriocins have been evaluated as the most promising class of antimicrobial peptides to be used as antibiotic substitutes in the field of animal and human medicine or for design and production of new antimicrobials (Sahl and Bierbaum, 2008). Particularly on animal trials, bacteriocin and bacteriocin-producing bacteria may be useful to improve animal nutrition and health through the manipulation of ruminal fermentation, the control of animal infections and the inhibition of enteric pathogens (Patra, 2011). Antibiotic therapy has been a valuable tool used in animal research, as growth promoters or therapeutic agents, and their efficacy and cost-effectiveness contribute to their popularity. Nevertheless, treatment of animals with antibiotics leads to antibiotic residue in the environment and veterinary products (Molinza *et al.,* 2003) as well as an increase in the frequency of resistance among bacterial species (Ochoa Zarzosa *et al.,* 2008). A number of strategies not dependent on antibiotics have been proposed to improve growth and feed conversion and novel strategies to reduce or eliminate animal pathogens have been tested by different research groups. The alternatives include bacteriocins, probiotic micro-organisms and bacteriophages (Bedford, 2000). Bacteriocins produced by different Gram-positive bacteria have been tested both in vitro and in vivo. The peptides that have been tested for livestock production differ in their physicochemical characteristics (Table 1) and spectrum of activity, but preliminary studies indicated that they might be a potential and effective alternative to classical antibiotics used in animal husbandry.

Modem husbandry systems usually involve large herds of young animals confined into limited spaces and fed similar diets. In order to maximize feed efficiency and maintain high levels of productivity, antimicrobial agents are often incorporated into animal feeds and water to improve feed digestion and prevent the occurrence of microbial diseases. Many substances affect animal performance indirectly, and the modification of the ruminal fermentation is suggested as the main effect of antimicrobials on ruminant animal.

Bacteriocin in Human Health

The rapid rise and spread of multi-resistant bacterial pathogens have forced the consideration of alternative methods of combating infection (Lee *et al.,* 2011). One of the limitations of using broad-spectrum antibiotics is that they kill almost any bacterial species not specifically resistant to the drug. Given such a broad killing spectrum, these antibiotics are used frequently, which results in an intensive selection pressure for the evolution of antibiotic resistance in both pathogenic and commensal bacteria (Torreblanca *et al.,* 1989). Once resistance appears, it is simply a matter of time and the intensity of human-mediated selection before human pathogens will acquire resistance (Lipuma *et al.,* 1990). Current solutions to this dilemma involve developing a more rationale approach to antibiotic use, which involves curtailing the prescription of drugs for anything other than bacterial infections, cycling through different drugs over a shorter time frame, and educating the public about the necessity of taking an entire course of antibiotics (Smajs *et al*, 1997). Bacteriocins provide an alternative solution. With their relatively

narrow spectrum of killing activity, they can be considered "designer drugs," which target specific bacterial pathogens. Given the diversity of bacteriocins produced in nature, it is a relatively simple task to find bacteriocins active against specific human pathogens. The development and use of such narrow-spectrum antimicrobials not only increases the number of drugs on the pharmaceutical shelf but, more importantly, extends their shelf life. This latter feature emerges because with a designer drug approach, each antibiotic is used infrequently, which results in a reduction in the intensity of selection for resistance. From an ecological and evolutionary perspective, the use of narrow-spectrum antimicrobials to address the current trend posed by multi-resistance bacterial pathogens makes quite a bit of sense. It leads to a reduction in the collateral killing of non-pathogen species that is commensal species, which in turn leads to a decrease in nosocomial infection levels. It also results in a reduction in the intensity of selection for antibiotic resistance. With so few species of bacteria killed by each designer drug, antibiotic resistance resulting from antibiotic use will evolve and spread more slowly (Pelczer, 1986).

Applications of Bacteriocins in Medicine

Bacteriocins have interest in medicine because they are made by non-pathogenic bacteria that normally colonize the human body. Loss of these harmless bacteria following antibiotic use may allow opportunistic pathogenic bacteria to invade the human body. As the narrow spectrum of bacteriocins produced by LAB represent an important limitation for the application of these bacteriocins as clinical drugs or as food preservatives (Acuña*et al.,* 2012), some examples of bacteriocins and their pharmaceutical applications are (a) the use of microcins derived from enterobacteria to control Gram negative bacteria (Gracia, *et al.,* 2010). Similarly to pediocin-like bacteriocins, microcins belonging to class IIa such asmicrocin V are linear polypeptides, and the removal of the leader peptide is the unique post translational modification that they undergo before being secreted by the cells. In order to obtain a peptide with a broader antimicrobial spectrum, recent works fused by asymmetrical PCR the required portions of genes encoding enterocin CRL35 and microcin V, namely mun A and cva C. The hybrid bacteriocin Purified from *E. coli* extracts, named Ent35eMccV, showed inhibitory activity against enterohemorrhagic *E. coli, L. monocytogenes*, and other pathogenic Gram positive and Gram-negative bacteria (Acuña *et al.,* 2012). In this field, several la ntibiotics are used in pharmaceutical applications (Van Kraaij *et al.,* 1999). Some have been used in dental caries treatment (mutacin-producing strain) (Hillman *et al.,* 2000) used to control vaginal microbiota with significantly reducing the adherence of the urogenital pathogen *Staphylococcus aureus* (Zárate and Nader-Macias, 2006). So far, nisin is the most promising in this medical field. The intravenous use of nisin has not been further developed since nisin shows a low stability at physiological pH. However, several protein-engineered derivatives of nisin Z have been generated in recent years that show improved stability and these or others may extend the medical application of nisin (Kuipers *et al.,* 1991; Severina *et al.,* 1998). Nisin was also applied in the treatment of respiratory tract infections. Some study reported the capacity of nisin to develop resistance in respiratory tract to prevent growth of resistant *Staphylococcus aureus* or *Streptococcus pneumonia* (De

Kwaadsteniet *et al.,* 2009). Also, recent work reported that Nisin F inhibits *Staphylococcus aureus* in the nasal cavities of immune suppressed rats (De Kwaadsteniet *et al.,* 2009). Many studies report the efficiency of nisin against several diseases responsible in digestive tract especially *Clostridium* species that can induce diarrhea: *C. botulinum*, *C. tyrobutyricum* and *C. difficile* (De Carvalho *et al.,* 2007; Irianto and Austin, 2002) and gastric ulcers: *Helicobacter pylori* (Kim *et al.,* 2000). Recently, the study of the therapeutic properties of nisin F in mice infected by *S. aureus* Xen 36 appeared to be promising to control the disease (Brandt, 2013). The resistance of spontaneous mutants to bacteriocins have also been reported, that may be related to changes in membrane and cell wall, such as alterations in the electrical potential, fluidity, membrane lipid composition and load or cell wall thickness or even a combination of all factors. These changes may occur following cell exposure to low concentrations of bacteriocins or as part of an adaptive response to some other stress. The resistance of *L. monocytogenes* to nisin is related to variation in fatty acid composition of cell membranes, reducing the concentration of phospholipids, hindering the formation of pores. The mechanism of resistance to subclass IIa bacteriocins appears to be linked to reduced expression of mannose permease of the phosphotransferase system (Kaktchman et al., 2012).

Bacteriocin in Aquaculture

Aquatic cultures are facing with the same problems with animal farming. They are continuously exposed to a wide range of microorganisms, some of which are pathogenic (Reilly and Kaeferstein, 1998). Many efforts were undertaken to prevent and control this dilemma: husbandry techniques and the use of vaccines and antibiotics (Smith, 2007). These methods can create several negative problems. They cannot prevent disease (husbandry techniques). Laborious, costly, and highly stressful to the animals (vaccines) and especially the selection for antibiotic resistant bacteria and active residues of the drugs remain long after use (Zhou and Wang 2012). An alternative approach to disease prevention in aquaculture is the use of bacteriocin-producing bacteria, BPB (Lauková *et al.,* 2003). It means use these bacteria as probiotic because in aquaculture, aquatic animal and microorganisms share the same ecosystem in the aquatic environment and it suggested that the interaction between the microbiota, including probiotics, and the host is not limited to the intestinal tract (Zhou and Wang, 2012). Many works reported that the administration of BPB as probiotic exclude competitively pathogenic bacteria through the production of inhibitory compounds, improve water quality, enhance the immune response of host species, and enhance the nutrition of host species through the production of supplemental digestive enzymes (Wang 2007). Most probiotics used in aquaculture belong to the lactic acid bacteria, of the genus *Bacillus*, to the photosynthetic bacteria or to the yeast, although other genera or species have also been mentioned. Many studies have reported promising results using a single beneficial bacterial strain as probiotic in the culture of many aquatic species (Zhou and Wang, 2012) but it is important to consider the possibility of using different species. The effect of probiotics, photosynthetic bacteria (*Rhodobacter sphaeroides*) and *Bacillus* sp. (*B. coagulans*), on growth performance and

digestive enzyme activity of the shrimp, *Penaeus vannamei*, was investigated and the results showed that the effects were related with supplementation concentrations of probiotics and thus use of a 10g/kg (net weight) supplement of probiotics in shrimp diet was recommended to stimulate productive performance (Wang, 2007).

Some study showed that nutrient and water enrichment with commercial BPB, designated Alchem Poseidon™ (a mixture of *Bacillus subtilis*, *L. acidophilus*, *Clostridium butyricum*, and *Saccharomyces cerevisiae*) significantly improved lysozyme activity, lowered levels of mucosal proteins and also improved survival after bacterial immersion challenge with *Vibrio anguillarum* (Taoka *et al.*, 2006). BPB has the potential to serve as an efficacious long term solution, as the administered bacteria become established in the host and/or the aquatic environment. Early attempts to use probiotic species in aquaculture usually employed BPB developed for terrestrial animals, which contained the facultative or obligate Gram positive anaerobes found in the GI tract, specifically of the general *Bifidobacterium*, *Lacto bacillus*, and *Streptococcus* (Gatesoup, 1999). Production of BPB specifically for the use in aquaculture is now a more popular approach, as these strains are more likely to establish in aquatic communities (Irianto and Austin, 2002). Bacteriocin producing strains should be developed to be more effective for aquaculture than the regular probiotic strains in the future.

Bacteriocin – active against Phyto Pathogens

The potential of gram – negative produced bacteriocin as a means of biological control in fighting plant pathogens has been investigated. Influenced by the prevalence of antibiotic – resistant phyto pathogenic bacteria (MacManus *et al.*, 2000) and growing health concern associated with chemical pesticide (Cook, 1993) to date several bacteriocin have shown promising results in carbing plant pathogens. Dipping plants in a suspension of a bacteriocin producing a virulent strain of *Ralstonia solanacearum* prevented tobacco with infection (Chen *et al.*, 2005). The incidence and severity of bacterial blight infection that causes leaf streak in rice was reduced by treatment with a nonpathogenic bacteriocin producing strain of *X. compestriproryzae* (Sakthirel and Mew, 1991). *S. plymithicium* is active against *Erwine amylovora.* This pathogen is the causative agent of fire blight, a costly disease to the apple and pear industry (Jabrae *et al.*, 2002).

A pyocin produced by *Pseudomonas syringae* and *P. coccaronei* inhibited the multiplication of *P. syringae sub sp. sarastamoi,* the causative agent of olive knot disease. This bacteriocin also effects the epiphytic survival of the pathogen on leaves and twigs of treated olive plants have been fused together generating active bacteriocin on active producing strains that have to date been considered safe for humans.

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