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A REVIEW ON ANTIMICROBIAL AGENT BACTERIOCIN: FROM LACTIC ACID BACTERIA (LAB)

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Abstract:

Bacteriocin is the proteinaceous agent produced by Lactic acid bacteria (LAB) Bacteriocin has wide range of antimicrobial activity which makes it important as food preservative, therapeutic agent. Bacteriocins were classified in several classes amongst which class I and class II were well studied. Increasing awareness of the consumers regarding diet and health and growing research regarding LAB enhance its importance as probiotic and a therapeutic agent

Keywords: LAB, Bacteriocin, antimicrobial activity, food preservative, therapeutic agent

Introduction:

Bacteriocins are the proteins possessing the antimicrobial activity. A great number of Gram negative and Gram positive produced bacteriocins (Todorov and Dicks, 2006).The antimicrobial activity of bacteriocin is restricted to the strains of species similar to the producing species (Perez *et al.*, 2014). A wide range of food grade lactic acid bacteria ribosomally synthesized the heat stable bacteriocins which have enormous prospective as food preservative and also targeting multidrug resistant pathogens (Perez *et al.*, 2014).

But an enormous range of bacteriocins which have wide range of antimicrobial activity are also recorded. LAB bacteriocins are naturally tolerant to high thermal stress and pH change over a wide range .Due to number of characteristics like these peptides are colorless, tasteless bacteriocins can be used as antimicrobial agents. In the history of bacteriocin there were no reports showing bacteriocin resistance developed by the target bacteria, indeed even at very low concentration bacteriocins promotes pore formation in the

membrane of target bacteria. However, the proteinaceous bacteriocins can be degraded by proteiolytic enzymes in human body which reduces probability of target bacteria to interact with the degraded fragments whichis the prime basis of development of antibiotic resistance. Bacteriocins are the primary metabolite with simple mechanism of biosynthesis as compared with the antibiotics, hence bacteriocins can be easily submissive by bioengineering to enhance their activity against the target microorganisms.

Lactic acid bacteria bacteriocins are normally considered as food grade as lactic acidbacteria are associated with food fermentation from ancient time.

Lactic acid bacteria (LAB):

Lactic acid bacteria (LAB) are the Gram-positive, non-spore forming rods, cocci and coccobacilli with a DNA composition of less than 35% G+C., non aerobic aerotolerent and capable to fermenting carbohydrates as energy source with lactic acid production (Zacharof and Lovitt, 2012). Although lactic acid can be produced as primary and secondary end product of fermentation by several genera of bacteria, LAB also produce diacetyl, hydrogen peroxide, organic acids and bacteriocin or bacteriocidal proteins during lactic acid fermentation beside lactic acid (Oyetayo *et al.*, 2003; Rodríguez *et al.*, 2003; Holzapfel *et al.*, 2001; Hirano *et al.*, 2003). Lactic acid bacteria includes major genera *Bifidobacterium, Enterococcus, Lactobacillus, Streptococcus, Pediococcus, Leucinostoc, Lactosphara, Lactococcus, Melissococcus, Aerococcus, Propionobacterium and Microbacterium*. LAB represent highest % of bacteria that produce probiotic properties (Carr *et al.*, 2002; Metchnikoff, 1908). Amongst the intestinal commensal *Lactobacillus*, *Bifidobacterium* are the predominant members which are commonly studied as probiotic bacteria (Kawai *et al.*, 2001).

Bacteriocins Classification:

The majority of LAB bacteriocins are small (<10 kDa) heat-stable, amphiphilic membranepermeabilizing cationic peptides. The LAB bacteriocins are classified into three major classes, viz. Lantibiotics, Non- lantibiotics and Bacteriocins. However their classification wascontinually revised during the last decade due to the extensive research realized (Rodriguez, 2000; Chen and Hoover, 2003).Most of the bacteriocins shows relatively modest adsorption specificity. The cell wall of Gram positive (+) bacteria allows passage of relatively large molecules. The anionic cell surface polymers like teichoic and lipoteichoic acids, which are part of the cellular wall, are important in the initial

antimicrobial activity; these peptides individually show slight or no activity against target organisms. Lactacin F and lactococcin G are members of this group (Paul Ross, 2002; Nes, 2013).

Class III: Bacteriocins:

Class III bacteriocins are mostly high molecular mass heat sensitive peptides of about (>30 kDa). Helveticin I by *Lactobacillus helveticus and* enterolysin produced by *Enterococcus faecium* are the example of this group (Paul Ross, 2002). They show the antimicrobial activity by disruption of cell wall and disorganization of cell membrane.

Table 1: Common bacteriocins produced by Lactic Acid Bacteria

Bacteriocin	Bacteriocin Producing Strain
Plantaricin S ß	Lactobacillus plantarum
Lactacin F	Lactobacillus johnsonii spp.
Lactoccin G	Lactococcus. lactis spp.
Lactocin 705	Lactobacillus. casei spp
Lactococcin MN	Lactococcus lactis var cremoris
Nisin	Lactococcus lactis spp.
Leucocin H	Leuconostoc spp.

Mode of action of bacteriocins:

Biological activity of bacteriocins exerted by its adsorption on the external surface of the target cells through the receptors present on it; followed by translocation in the target cell. At neutral pH the majority of LAB bacteriocins are cationic peptides with presence of lysine, arginine and histidine, hydrophobic in nature with the amino acids viz. alanine, valine, leucine, isoleucine, proline, methionine, phenylalanine and tryptophan and amphiphilic, containing 20 to 60 amino acids (Cotter *et al.*, 2005). These properties bacteriocins play important role during their action on cytoplasmic membrane, where the cationic bacteriocinsbind to negatively charged phospholipids that make up part of the membrane of sensitive cells whereas allocation of the bacteriocins all over the cytoplasmic membrane is supported by its amphiphilic nature of bacteriocins (Thomas *et al.*, 2001). Class I and Class II bacteriocins reveal their antimicrobial activity at acidic and neutral pH, however they are sensitive to proteolytic action of pancreatic and gastric enzymes like

interaction of anionic bacteriocins produced by Gram positive (+) bacteria. Adsorption of lactic Acid Bacteria bacteriocins to it surface and other Gram positive bacteria is pH dependent. It shows significant antimicrobial activity lower pH (pH< 5). There might be amino acid sequence homologies amongst the mature peptides, N- terminal leader regions of the peptide as well as the proteins associated in secretion and processing of bacteriocin amongst the classes of bacteriocins (Cleeveland, 2001).

Class I: Lantibiotics:

The Class-I lantibiotics, are a class of antimicrobial peptides which comprise of unusual polycyclic thioether amino acids and unsaturated amino acids like lanthionine (Lan) or methyllanthionine (MeLan) and dehydroalanine and 2-aminoisobutyric acid respectively. Lantibiotics are further categorized in two types; Type A includes screw shaped, expanded, cationic, amphipatic, flexible molecules with molecular mass between 2 to 4 kDa. Mode of action of lantibiotics is membrane depolarization and pore formation in the membrane of sensitive species. Nisin and lacticin 3147 are the major representatives of this group; nisin produced by *Lactococcus lactis*, active against Gram positive bacteria, such as LAB, *Listeria SP*, *Micrococos SP* and spore forming bacteria like *bacillus SP* and *clostridium SP*. Whereas Type B lantibiotics, are globular in structure, either anionic or they have no net charge with molecular mass ranging between 2 to 3 kDa. Type B lantibiotics show antimicrobial action against target organism through interference in cellular enzymatic reactions (Cleeveland, 2001; Deegan *et al.*, 2006).

Class II - Non-Lantibiotics:

Non-Lantibiotics the Class II bacteriocins are heat stable peptides with molecular mass of < 10 kDa. Non-Lantibiotics peptides lack lanthionine. Non- lantibiotics are categorized in two subclasses. Subclass II a, includes non lantibiotics with an N-terminal consensus sequence Tyr- Gly-Asn-Gly-Val-X-Cys which is ordered into a S- shaped antiparellel β- sheet that are stabilized by disulfide bond like in pediocin or listeria active bacteriocins (Chen and Hoover, 2003).

Bacteriocins belong to subclass II a show 40%-60% of homology when the corresponding amino acid sequences are aligned. Non-Lantibiotics subclass II a bacteriocins are synthesized with a N terminal leader peptide which is cleaved during process of secretion by proteiolytic action usually after a double glycine residue for example like pediocin PA-1, sakacin A (Daw and Falkiner, 1996). Subclass II b refers to two-peptide bacteriocins it means that requires two peptides to work synergistically in order to have an

proportional to its population. Actively growing cells are more sensitive to bacteriocins; whereas the cells which are not actively growing, endospores might show resistance upto certain extent as food processing procedures stimulate germination of these endospores which enhance the bacteriocin effectiveness against the spores. Production of bacteriocin by the organism is associated with its growth, hence the factors (nutrients, growth inhibitors) affecting cell growth also its production and ultimately its effectiveness against the target organism. For example the sausage ingredients and the food additives, with an exception of nitrate considerably inhibit enterocins A and B production by *Enterococcus faecium* CTC492 (Oliveira *et al.*, 2005).

Nisin:

Nisin is the member of Class I lantibiotic, most extensively utilized bacteriocin. It was approved by FDA for its utilization in various products viz. cheese spreads, cheese, heat treated chill storedsoups by 1988. Nisin shows antimicrobial activity against Gram positive food pathogen and spoilage responsible bacteria like *Staphylococcus aureus* and *Listeria monocygenes*. Nisin is a thermostable protein it is stable at 121°C, however extended temperature exposure may abate its thermostability particularly over a pH range 5 -7. It is resistant to several proteases viz. trypsin pepsin, carboxypeptidase etc. but sensitive to α-chymotrypsin. Hence it could be utilized as food additive worldwide as E234 with GRSA status (Cleeveland *et al.*, 2001; Deegan, 2006, Mattick and Hirsch, 1947) designated the name 'Nisin' for the bacteriocin from *Lactococcus lactis* derived "*N* inhibitor substance" as *Lactococcus lactis* formarly classified as Lancefield serological N *Streptococcus* (Dicks *et al.*, 2011). *Nisin* is composed of 34 amino acids including several unusual amino acids as result of post translational modifications. Structure of nisin consists of a lanthionine and four ß-methyllanthionines (Rodriguez *et al.*, 2000). The precursor peptide of nisin was ribosomally synthesized and then subjected to modification. The mature nisin is obtained as result of removal of n-terminal leader sequence. A nanomolar concentration of is capable of showing antimicrobial activity against target organism (Cleeveland, 2001).

Nisin: Mode of action:

Nisin and subgroup Lantibiotics interact with the anionic lipids in Gram positive bacteria membrane and diffuse through it. The formation of pores in the lipid membrane was carried out through interaction of nisin with the Peptidoglycan precursor lipid II it inhibit Peptidoglycan synthesis (Wiedemann (2001). Mostly the amphiphilic of nisin type sub group Lantibiotics owing to itselongated cationic peptides disturb integrity of thee

trypsin, chymotrypsin and pepsin, beside this these bacteriocins endure extremes pH, temperature and salinity.

The majority of LAB bacteriocins exert their antimicrobial against the sensitive bacteria through formation of pores in the cell membrane and dissipating the proton motive force. When bacteriocins come in contact with cell membrane of the target cell; bacteriocin bind to the cellsurface of the target cell through N- terminal in the forms a sheet like structure. However the hydrophobic C- terminal into the hydrophobic core of the target- membranes. And pierce cell further binds to the mannose phosphotransferase permease which results in membrane leakage. Immunity proteins which protect the target cells from pediocin-like bacteriocins bind to this bacteriocin-permease complex and stop bacteriocin-induced membrane-leakage. While the two- peptide bacteriocins penetrates through the target cell cytoplasmic membrane as a helix-helix structure; they may also bind the integral membrane proteins (Yusuf and Hamid, 2013).The outer membrane of Gram negative bacteria protect them from the antimicrobial activity of LAB bacteriocins.

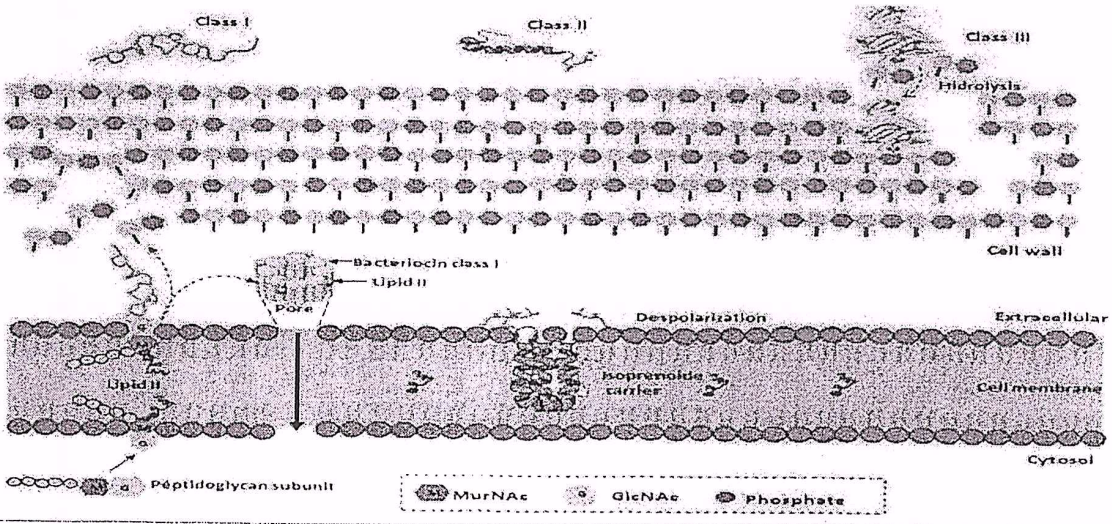

Figure 1: Mode of action of LAB bacteriocins. Adopted from (Álvarez-Cisneros *et al.*, 2011)

Certain Class I bacteriocins shows twofold mode of action, along with pore formation intarget cell membrane it could bind to lipid II; which results into blockage of cell wall synthesis and death of cell. The amphiphilic Class II bacteriocins peptide easily get inserted into the membrane of the target organism and results in depolarization and death of the target cell (Oliveira *et al.*, 2005).

Various factors affect the effectiveness of bacteriocin on target cell like the load of target cells, concentration of bacteriocin required for destruction of target cell is directly

since the food functionality and its nutritive quality .These non thermal treatments help in destabilization of cell membrane and consequently obstructing the essential cell functions (Deegan *et al.*, 2006). These non thermal techniques extremely effective along with lower concentration of bacteriocins else alone these techniques might not financially feasible. In addition to inhibit growth of contaminants responsible for food poisoning and spoilage bacteriocins also enhance the food quality and sensory properties like *Enterococcus faecium* *sp.* FL 3, bacteriocin BacFL31 inhibit the spoilage microorganisms responsible for oxidative rancidity as well as *Listeria monocytogenes* and *salmonella typhimurium* and retained the pH at low. Along with this it also retain the sensory properties like odor, color, texture at suitable levels for long time, thus improve the shelf life of the meat (Turubatovic, 2013).

Bioactive packaging is one of the more application of bacteriocins contributes to improve the shelf-life of food.. Bioactive packaging is method to protect the food from the external contamination during storage and handling. The steady release of bacteriocins from a packaging film on the food surface is more beneficial as spraying of bacteriocin on food products may not be sufficient as its activity may diminish as result of inactivation of interaction with food components. Its low concentration might not sufficient to be against upto interior of the food product. The bioactive packaging films can be used by various methods, the bacteriocins cab be directly added in the biodegradable protein films preparation from soybean, corn through heat press and casting or the bacteriocins can also be coated or adsorbed on the polymer film like methylcellulose, polyethylene, ethylene, vinyl acetate, polypropylene, polyamide, polyester acrylics and polyvinyl chloride (Deegan *et al.*, 2006). Primarily bacteriocins are employed to control food spoilage and related food poisoning however bacteriocins can be utilize as innovative medicinal treatment against pathogens in human body as bacteriocins have different approach of antimicrobial activity over antibiotics (Dicks *et al.*, 2011). Beside this bacteriocins have another benefit over antibiotics that as antibiotics due to their broad spectrum activity inhibit commensal organisms along with the target pathogen while due to comparatively narrow spectrum bacteriocins exhibit its antimicrobial activity against target organism (Riley and Wertz. 2002).

Bacteriocins have desirable properties like high activity even at nanomolar range. high specificity and mechanism of action which prove it to be used as medicine (Van Heel *et al.*, 2011). Multidrug resistance is the major problem in currant scenario which led to investigate an alternative to overcome the infection. As bacteriocins have specific mode of action as that of usual antibiotics, represent them as potential substitute for the antibiotics. Bacteriocins can inhibits multidrug-resistant Pseudomonas aeruginosa.

energy transducing membrane. It was demonstrated that nisin like Lantibiotics stimulates rapid efflux of ions, amino acid or nucleotides, resulted in depolarization of membrane results in instantaneous termination of all biosynthetic processes (Wiedemann, 2001).

Nisin seems to align parallel to the surface of the membrane, the C terminus of nisin inserted in the membrane causes inter monolayer contact of phospholipids, forming pores according to the wedge like model. The ephemeral interruption of the phospholipid of the membrane structure acquired locally during the membrane permiabilization. According to the wedge-like model, the pore formation involves a proton motive force driven by co insertion of lipids and nisin C terminal domains. Such multiple inserted nisin molecules might leads to a great local disturbance of the lipid protein pores. Such structures are inherently not stable owing to the hydrophobic forces, which are the forceful, the rearrangements of the lipids into their usual bilayer organization (Moll, 1998).

Applications of Bacteriocins:

Bacteriocins have been extensively exploited as food preservative in the food industries in the current scenario particularly in dairy, egg, meat and vegetables product industries. It has reported that nisin A and Nisin Z have potent antimicrobial activity against food spoilage and food poisoning causing organisms. Moreover the unique feature of nisin over the other LAB bacteriocins that nisin is has been legitimately utilized and accepted globally in food industry (Moll *et al.*, 1999; Deegan *et al.*, 2006). Various techniques viz thermal treatment like pasteurization, sterilization, use of low pH and water activity environment for example acidification and dehydration or incorporation of preservatives like antibiotic, organic acids viz acetic acid, lactic acid, propionic acid etc or their salts viz sodium acetate, sodium lactate. Beside this these physical chemical preservatives there is increasing requirement for natural microbiologically safe product to ensure the health benefits as the thermal or chemical preservation may lead to deterioration of nutrient (Deegan *et al.*, 2006).

Bacteriocins can be introduced in the food products during their manufacture may be as a starter culture or co starter culture or may be as a already fermented product which contains the desired bacteriocin else as a purified bacteriocin as one of the component during the food processing (Paul Ross, 2002). Bacteriocins are competent against Gram positive bacteria as a food preservative but to be active against Gram negative organisms bacteriocins needs to be used along with the other technologies which facilitate the disruption of cell membrane for its effective antimicrobial activity (Daw and Falkiner, 1996). The non thermal treatment like hydrostatic pressure, pulsed electric field are helpful

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