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Exploration of Bacteriocins as Potential Food Preservatives

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ABSTRACT

Over the last couple of decades demand for healthy food has increased because people have become more health conscious. They inclined towards less processed food which does not contain harmful chemical preservatives as they cause serious health problems. Bacteriocins are ribosomally synthesized extracellular bioactive peptides, known for its antimicrobial activity, has no side effect on human health. It has antagonistic to other strains but contain self immunity mechanism. Nisin produced by lactic acid bacteria, inhibit not only closely related species but also effective against food-borne pathogens and specially gram positive spoilage bacteria besides gram negative bacteria. Many bacteriocins have been tested as preservative in a wide range of food products which include fruits, vegetables, seafood, dairy, fermented & meat products. FDA has approved these bacteriocins safe for human consumption. A large number of bacterial species produce these antimicrobial compounds like *lactococcus, streptococcus, lactobacillus, enterococcus, carnobacterioum* etc. A bacteriocin named nisin is commercially used in 50 countries all over the world due to its various food applications. Its production technology can be improved by using new strains and immobilized with bio plastic for future food packaging industry which is growing 3 % annually of 24 billion UDS annual market in 2015.

Keywords: - Bacteriocins, Preservation, Nisin, Food Spoilage.

INTRODUCTION

Food preservation has become a major issue with the increasing demand of high quality safe food due to increase in our ever growing population. Global food market has also seen tremendous growth in packaged food retail market in 2010 & 2015 and recorded the world wide business 1.95 and 2.14 trillion USD respectively. [1] Consumer demands for faster, healthier and ready-to-eat products that strongly increased the need of more natural preservatives instead of chemical preservatives. [2] The chemical preservatives used in food to

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inhibit the microbial growth lead to serious health problems. Several physical and chemical treatments employed to increase the shelf-life of food but these treatments have some ill effects on human health so bacteriocins offer an alternative to chemical preservatives as they are safe antimicrobial compounds.[3] A diverse range of bacterial species produce this antimicrobial compound, known as bacteriocin which is used as natural food preservative. Bacteriocins are proteinaceous antimicrobial substances which are ribosomally synthesized, extracellularly released bioactive peptides. [4] These bacteriocins are generally regarded as safe (GRAS). [5] These are produced by one bacterial strain which are antagonistic to other strain and species, and the producer strain is protected from its own bacteriocin by immunity mechanism. [6] These antimicrobial compounds are first described in *Escherichia coli* termed as colicins. [7] Most important genera which produce bacteriocin include *Lactococcus, Streptococcus, Lactobacillus, Enterococcus, Carnobacterium*. Among the metabolites of lactic acid bacteria, bacteriocins are the only antimicrobial substances which have the utmost capacity to inhibit growth of food spoilage and pathogenic organisms. [8] There are large numbers of bacteriocins produced by different strains of lactic acid bacteria like lacticin produced by *lactococcal* sp., pediocin from *pediococcus* sp., sakacin 674 from *lactobacillus* sake, helveticin from *lactobacillus helveticus*, entrolysin from *enterococcus faecalis* . [9] Bacteriocins could be categorized as antibiotics but they are not. The major difference between bacteriocins and antibiotics is that bacteriocins restrict their activity to strains of species related to the producing species and particularly to strains of same species. On the other hand antibiotics have a wider activity spectrum and even if their activity is restricted this does not show any preferential effect on closely related strain. [10] Some bacteriocins produced by lactic acid bacteria such as nisin, inhibit not only closely related species but also effective against food-borne pathogens and many other gram positive spoilage bacteria. [11] Many bacteriocins have been tested as preservative in a wide range of food products which include fruits, vegetables, seafood, dairy & meat products. [12 ,3] Bacteriocins usually have low molecular weight (rarely over 10 KDa) they undergo post-translational modifications and can be easily degrade by proteolytic enzymes especially by the proteases of the mammalian gastrointestinal tract which makes them safe for human consumption. [10] Among the bacteriocins, nisin is the only bacteriocin to have found a widespread application in the food industry. It is active against many gram positive bacteria

including *listeria* sp. It prevents the growth of germinating *bacillus* and *clostridium* spores and through the addition of calcium chelator, it is active against some negative bacteria. Several bacteriocins from LAB extend potential applications in food preservation, thus help foods to be naturally preserved and richer in organoleptic and nutritional properties. [13] Toxicity, processing, stability, broad spectrum inhibition activity, effect on food properties and a thorough understanding of their biochemical and genetic properties of some of the important parameters considered during the application of bacteriocin in food. [14] The last twenty years can be regarded as the "golden era" for the discovery of novel bacteriocins produced by lactic acid bacteria. [15] Today, nisin is approved as safe for use in food in over 50 countries all over the world. It was suggested that the combined lack of awarerness of what bacteriocin can achieve in food system and the lack of enthusiasm to move away from existing food preservation techniques is the reason for the under-utilization of bacteriocin in food industry. [6] Various food processing techniques like use of heat, use of chemical preservatives, low temperature, modified atmosphere etc. are used to prevent food spoilage but these methods have their own drawbacks. [16] Some of the harmful effects of chemical preservatives showed in the table1.

Physical, chemical and biological properties of bacteriocins

Bacteriocins are ribosomally synthesized antimicrobial peptides produced by microoorganisms belonging to different eubacterial taxonomic branches. [17] The production of small antibiotic peptides is a common defense strategy against bacteria that is displayed not only by microorganisms, but also by animals and plants [17] Maganins, cecropins and defensins are animal [18 ,19] , and thionins are plant [20] antimicrobial peptides. Protease sensitivity is a key criterion in the characterization of an inhibitor as a bacteriocin. Since bacteriocins are by definition proteinaceous substances, they are generally inactivated by an array of proteolytic enzymes (trypsin, α-chymotrypsin, pepsin, proteinase K, etc.). Moreover, the pattern of protease sensitivity may indicate the uniqueness of an isolated bacteriocin, since bacteriocins of different bacteria vary in sensitivity to proteolytic enzymes. [21] Conversely, the same pattern of protease sensitivity coupled with an identical spectrum of activity may indicate multiple isolations of the same bacteriocin producer. In addition, the sensitivity to proteolytic enzymes of gastric and pancreatic origin is very interesting with respect to the application of bacteriocins as biological preservative in foods and feeds, since it means

that their ingestion will not affect the microbial flora of the gastrointestinal tract. [21] However, some bacteriocins seem to be insensitive for certain protease. It is possible that these bacteriocins contain only a minor proteinaceous component; several bacteriocins contain a carbohydrate, lipid or phosphorous moiety. The presence of such a non-proteinaceous moiety can be verified by the sensitivity of these bacteriocins glycolytic (α-amylase), lipolytic (lipase) and phospholipolytic (phospholipase) enzymes. [21] Bacteriocin 466 [22] and lactocin 27 [23] are typical examples of such lipocarbohydrate protein complexes, whereas caseicin LHS [24] and leuconocin S [25] behave as glycoproteins. In addition, some bacteriocins contain unusual amino acids (lantibiotics) or several cross-linkages, making the elucidation of their primary structure rather difficult. As an example, the post-translational formation of thioether amino acids during lantibiotic biosynhesis results in the formation of intramolecular rings by monosulfur bridges. [26] Such ring structures will result in blank cycles during the automated sequencing by Edman degradation. [27] The molecular mass of bacteriocins produced by lactic acid bacteria may vary considerably, ranging from small peptides (e.g. lactocin 481, 1700 Da) to protein - protein and protein - lipid aggregates and macromolecules with a molecular mass in excess of 200 000 Da (e.g. lactocin 27, lactacin B, lactacin F, helveticin J). These aggregates can be disrupted by treatment with detergents or urea; the disruption often results in monomers which are more active than the native bacteriocin complex. For example, polyacrylamide gel electrophoresis of lactocin 27 in the presence of SDS showed that the active moiety was a protein with a molecular mass of 12 400 rather than 200 000 as determined by size-exclusion chromatography. [23] The stability of bacteriocin preparations has often been shown to decrease significantly with increased purification. Also, bacteriocins differ largely with respect to their sensitivity to inactivation by changes in pH and temperature. Most of the bacteriocins produced by lactic acid bacteria are only stable at acid and neutral pH and are inactivated at a pH above 8.0 (nisin, pediocin AcH, leucocin A-UAL 187). Heat resistance is a major characteristic of many bacteriocin produced by lactic acid bacteria and can vary significantly, ranging from 60˚C or 100˚C for more than 30 min (e.g. lactocin 27, lactocin S, carnobacteriocins A and B) to autoclaving at 121˚C for 15-20 min (e.g. lactacin B, lactacin F, nisin, etc.). Such heat stability is due to the formation of small globular structures and the occurrence of strongly hydrophobic regions (e.g. lactacin F,

lactococcin A, nisin), stable cross-linkages (e.g. nisin, lacticin 481, lactocin S), a high glycine content (e.g. diplococcin, lactacin F, lactocin 27, lactococcin A), etc. [21]

CLASSIFICATION

Bacteriocins of Gram negative bacteria

Bacteriocins produced by Gram-negative bacteria can be divided into two groups, namely the colicins and the microcins. Colicins are large (25- to 80-kDa) bactericidal proteins, the production of which is induced by conditions triggering the SOS system. [28] Colicin-like proteins produced by *Pseudomonas* species are called pyocins. One example is puticidin, a pyocin of 276 amino acid residues produced by *P. aeruginosa*. [29] Microcins are smaller (<10 kDA) and their synthesis is not SOS-inducible. [30] Microcins are divided into the following two groups:

Class I microcins are post-translationally modified peptides with a mass of less than 5 kDa with activity against intracellular targets. Members of this group are microcins B17, C7 and J25 [31] Microcin J25, produced by *Escherichia coli*, was originally classified as a Class V bacteriocin [32] based on a predicted circular nature. [33] Discrepancies in the NMR data and unexpected results after thermolysin digestion led to a re-examination of the published data. In the current model the N-terminus of this 21-residue peptide is covalently linked to the glutamate at position 8 creating a side-chain-to-backbone cyclization. The C-terminal region is threaded through the loop created by this cyclization event thereby effectively shielding it from protease attack. Microcin J25 appears to be a molecule with a lassoed tail. [34-36] Class II microcins are unmodified peptides with a molecular mass between 8 and 10 kDa. They exert their function through membrane depolarization. Members of this group of bacteriocins, produced by Enterobacteriaceae, are microcins E492, L and colicin V. [31] They have several features in common with Class IIa bacteriocins from Gram-positive bacteria such as their sizes, double-glycine-leader-directed secretion via an ABC transporter and relative high hydrophobicity. [28]

Bacteriocins of Gram-positive bacteria

Bacteriocins of Gram-positive bacteria vary a lot in make-up and general structure. They have features in common such as heat stability, protease resistance and the fact that most run faster through an SDS-PAA gel than predicted on the basis of their sizes. Available 3D-structures of some bacteriocins show that some of them are tightly folded peptides. Internal cross-linking supports the stability of many of these peptides.

Bacteriocins produced by Gram-positive bacteria are grouped in the following classes on the basis of their internal cross linking and primary structures amino acid sequence, mode of action, heat tolerance, biological activity, presence of modified amino acids and secretion mechanisms. [37] This classification is readily accepted and all future classification are slight modification of this scheme.

Class I bacteriocins

This class of bacteriocins are called lantibiotics. They are small (<5KDa) , heat stable peptides which acts on the membrane structure. They contain 19 to more than 50 amino acids. These are post translationally modified peptides [38] and have unsual amino acids such as lanthionine, α-methyllanthionine as well as dehydroalanine and 2-aminoisobutyric acid. They are produced by lactic acid bacteria to attack other gram positive bacteria. [39] Nisin, subtilin, cytolysin and variacin 8 are some examples of this group. [40-41] This class is further subdivided into two subclasses, Class Ia and Class Ib based on their chemical structure and antimicrobial activities. [42] Class Ia bacteriocins are elongated , flexible, screw-shaped and positively charged peptides. [43] They generally act on the cytoplasmic membrane of target species by forming pores on the membrane. Nisin is the example of this group. Class Ib bacteriocins are globular small, rigid peptide that have negative charge or no net charge. They exert their action by interfering with essential enzymatic reaction of sensitive bacteria. [43]

Class II bacteriocins

These bacteriocins are called non-lantibiotics because they do not contain the unusual amino acid lanthionine. These peptide are smaller than 10 KDa [44] unmodified or minimally modified and heat stable bacteriocins. [45] This class is subdivided into three subclasses based on amino acid sequence alingment. Class IIa include pediocin that have antilisterial peptide having a consensus N-terminal sequence Tyr-Gly-Asn-Gly-Val-Xaa-Cys and two cystein forming a S-S bridge in the N-terminal half of the peptide. Pediocin PA-1 and sakacin P are the examples. Class IIb comprises bacteriocins that are composed of two different peptides that needs to be fully active. The sequence of primary amino acid of peptides are different. Lactococcin G and Plantaricins EF are examples of this group. Class IIc include thiol-activated bacteriocins that are secreated by general sec system. [46] This class include circular bacteriocins, divergicin A and acidocin B.

Class III bacteriocins

These are large more than 30 KDa, heat labile peptides. [47] Helveticins S & V, lactocins A &

B, acidofilicin A are some example of this group.

Class IV bacteriocins

These bacteriocins are complex proteins that require carbohydrates and lipid moieties for activity. [48] Plantracins S and Leuconocin S are major example of this group of bacteriocins. [49]

Class V bacteriocins

Circular, head-to-tail ligated bacteriocins. These bacteriocins (e.g. gassericin A, circularin A and enterocin AS-48) form a peptide bond between the processed N-terminus and the C-terminus of a precursor peptide. [50] The head-to-tail ligated bacteriocins are here classified as a novel class, Class V, instead of Class IV to which they were initially assigned. Classification on the basis of shared features is sometimes inadequate as some bacteriocins can be placed in more than one sub-class: e.g. enterocin P and listeriocin (Class IIc) could also be classified as Class IIa and enterocin L50 while aureocin A70 (Class IId) could also be placed in Class IIb.

MODE OF ACTION OF BACTERIOCINS

LAB bacteriocins work via different mechanisms to exert an antimicrobial effect, the cell envelop is generally the target. [66] Nisin has a broad spectrum activity against gram positive bacteria and it also inhibit the growth of Bacillus spp. and Clostridium spp. spores. [40] Nisin has a dual mode of action; it acts on the cytoplasmic membrane as well as the cell wall biosynthesis. It acts on the cytoplasmic membrane of gram positive bacteria which cause lesions on the membrane. [67] The formation of pores disrupts the proton motive forces which ultimately interfere with cellular biosynthesis. This results in leakage of ions and hydrolysis of ATP which cause collapse of membrane resulting in cell death. [68, 2] Nisin also interfere with the cell wall biosynthesis. [69-70] This phenomenon is mediated by the ability of nisin to bind lipid II, a peptidoglycon precursor thus inhibiting cell wall biosynthesis. [71] Nisin binds to lipid II by two of its amino terminal rings which forms a complex of eight lantibiotic bacteriocins and four lipid II to initiate the process of membrane insertion and pore formation which lead to cell death. [6, 72] There are some other lantibiotics that form pores which include lacticin 3147, subtilin, Pep5 and epidermin. [73] The lantibiotic Pep5 has also nisin like concentration-dependent mode of action, which is also affected by physiological conditions such as ionic strength, pH, and temperature as well as by growth phase of the target cells. [74] These lantibiotics inhibit the biosynthesis of DNA, RNA, protein and polysaccharides leading to speculation that treated cells in a

way that they have no longer sufficient amount of energy to carry out biosynthetic processes. [75]

Sonorensin, a bacteriocin which belongs to the subfamily heterocycloanthracin, found to be very effective against biofilm of *Staphylococcus aureus.* When the cytoplasm membrane is permeable Ortho-Nitrophenyl-β-Galatoside (ONPG), which is a non membrane permeablize chromogenic substrate , it enters the cytoplasm and is degraded by β-galactosidase, which produce O-Nitrophenol that shows absorbance at 405 nm. [76] Sonorensin induced an increase in the permeability of *S. aureus* cytoplasmic membrane over time and in case of nisin (at the same concentration) , which has also the same MIC value against *Staphylococcus aureus* produced similar results of permeability. This suggested that sonorensin could permeabilize the cytoplasmic membrane of *Staphylococcus aureus.* Class II bacteriocins which include plantaricin, lactococcin G, thermoptilin 13, lactocin F and lactocin 705 [77-78] furnish the membrane of sensitive bacteria permeable to small molecule. Lactococcin G permeabilizes the target-cell membrane for a variety of monovalent cations such as K^+, Li^+, Cs^+, Na^+ , it does not permeabilize the cell membrane for divalent cations such as Mg^{2+} and for anions such as phosphate. [79-80] There is one more group of bacteriocins known as circular bacteriocins, group of N-to-C terminally linked antimicrobial peptides which are produced by gram positive bacteria of phylum *fermicutes.* These have generally broad spectrum activity and are known for their pH and thermal stability as well as for resistance to many proteolytic enzymes. A few circular bacteriocins (enterocin AS-48, Leucocyclicin Q and Lactocyclin Q) show their antimicrobial activity against *e.coli* but significantly higher concentration is required in comparison to gram positive bacteria. [81-82] Studies have shown that the circular bacteriocin, enterocin AS-48 forms non-selective pores in liposomes which leads leakage of ions and low molecular weight compounds. Carnocyclin A show its antimicrobial activity against gram negative bacteria, when the integrity of cell wall is disturbed by EDTA, a metal chelating agent. [83] Carnocyclin A forms pores in lipid membrane but unlike the pores which is formed by enterocin AS-48 , because the pores of carnocyclin A are anion selective and voltage dependent. [84] Other bacteriocins such as pediocin JD also have been reported to cause decandence of the membrane potential and increase the membrane permeability to ions which leads to disintegrate the proton motive force. [85-86]

PRODUCTION, PURIFICATION AND CHARACTERIZATION OF BACTERIOCINS

The course for discovery of bacteriocins include various steps. It begins by screening for bacteriocin producing strains from ample number of isolates. These strains are then grown in well suitable medium in order to produce highest amount of bacteriocins which are later purified by various purification mechanisms leading to classification and characterization. Basis of strain choosen to be screened for bacteriocin activity, it may be environmental isolates or formerly purified and distinguished strains. Lactic acid bacteria secretes numerous substances that have antimicrobial activity including hydrogen peroxide, lactic acid, aldehydes and ketones. [87] The antimicrobial activity due to these substances must be eliminated before detection of antimicrobial activity due to bacteriocins can be determined. The methods used for the detection of bacteriocin activity are based on classical antibiotic detection methods, in which the antimicrobial activity of the suspected bacteriocin is tested against a bacteriocin sensitive indicator strain. Direct detection methods are usually used for initial screening and usually involve growing the colonies of potential producer strain on the agar surface seeded with cells of a sensitive strain. During incubation the potential producer cells and indicator strain grow simultaneously and any antimicrobial activity due to bacteriocin like inhibitory substances is indicated by the presence of zones of inhibition around the producer cells after incubation. Other commonly used antagonism assay methods include the spot-on-the-lawn assay [88] , agar well diffusion assay. [89-90] The next step after selection of the bacteriocin producing strain, involves growing the strain in an appropriate medium under optimum conditions for maximum bacteriocin production. The bacteriocin is extracellularly secreted into the medium during growth of the producer strain. The bacteriocin is then purified and separated from the contents of the medium in a series of steps, followed by identification at the molecular level. Since the bacteriocins are heterogeneous in nature, therefore, the purification strategy varies for each bacteriocin.

Most of the bacteriocins are low molecular weight, cationic, and contain hydrophobic amino acid residues; therefore, these properties are usually exploited to purify bacteriocin to homogeneity. [14] Commonly used purification schemes include cation exchange chromatography and hydrophobic interaction chromatography followed by Reverse-Phase High Pressure Liquid Chromatography (RP-HPLC) as a final step.

Gel filtration chromatography and other steps may also be included to further purify some of the bacteriocins, but RP-HPLC is usually the final step in the purification scheme which purifies the bacteriocin to homogeneity and separates it from any remaining contaminants. [91] The purity is then confirmed by running the purified fraction in a SDS-PAGE gel, followed by in-gel activity testing. The presence of a single active band in the gel confirms that the bacteriocin has been purified to homogeneity, and the primary structure is then determined using techniques such as N-terminus sequencing and mass spectrometry. Alternative purification schemes have been suggested to either reduce the time or the number of purification steps thereby reducing the losses in activity. Novel methods have been developed which selectively separate bacteriocins from contaminants in minimal steps. One such novel procedure was developed by [92] , which exploits the cationic nature of bacteriocins. It is based on the principle that cationic bacteriocins are selectively adsorbed to the cells of the producer strain at neutral pH (maximum adsorption of about 90% at pH 6.0), and are desorbed at pH of 2.0 (about 99% desorption). The procedure involves the production of bacteriocin in growth medium followed by heating the medium to about 70 ºC to kill the cells. The pH of the medium is then adjusted to 6.0 which results in the adsorption of the bacteriocin to heat-killed cells. The cells are then removed from the production broth and then resuspended in a small volume of saline buffer at pH 2 at a temperature of 4 ºC. This results in desorption of cationic bacteriocins from the cells into the buffer. The bacteriocin containing buffer is then dialyzed after the removal of cells, followed by lyophilization. The resulting bacteriocin is highly purified and may require only one more step such as RP-HPLC to purify bacteriocin to homogeneity. Yang *et al*. in 1992 were able to recover more than 90% of the bacteriocin in the case of Pediocin AcH, nisin and leuconocin Lcm1.

APPLICATIONS OF BACTERIOCINS

Food preservation

Many bacteriocins of LAB and some other antimicrobial substances are used to start cultures or co-cultures in food production processes for increasing shelf life and nutritive value. [93] Bacteriocins are natural food additives due to presence of bacteriocins producing bacteria in large number of foods such as yoghurt, cheese, milk fermented meat products etc. [94-95] In bio-preservation, nisin is the first antibacterial peptide produced by LAB. [96] It is also the first commercial bacteriocin used for food preservation which is

marketed as Nispalin®. Nisin and pediocin are the two bacteriocins which are used as food additive in 50 countries all over the world. Few names of countries which use nisin are listed in the table 3.

Pediocin is also used commercially which is marketed as Alta® 2341 which is very effective against *Listeria monocytogenes* in meat products. [93] In traditional European Cheese, the milk used in the manufacturing process is easily contaminated by the animal excrements. The bacteriocin producing Enterococci used as starter culture or co-culture is effective for reducing microbial contamination from cheese. [98] Enterocin AS-48 produced by Enterococcus is used in fruit and vegetable juices, cider and canned vegetables for the inhibition of microbial contamination. Enteriocin EJ 97 and CCM4231 are also used in zucchini puree and soy milk for reducing bacterial count.

Bacteriocins can be used with hurdle technology to improve food safety. One microorganism *Staphylococcus aureus* is capable of producing enterotoxins which are responsible for Staphylococcal food poisoning. It is one of the most prevalent causes of gastroenteritis disease. Bacteria found in the mucous membrane and skin of warm-blooded animals. It causes mastitis named disease when infecting the cow, buffaloes and other mammals. The raw milk becomes continuously contaminated at level which may reach 10^5 CFU ml^{-1}. In this case enterocin with high pressure treatment (which is a non thermal method of food preservation used for large number of food products) is used to inhibit the growth of *S. aureus* in raw milk and cheese. Cheese made up of milk which is not inoculated with bacteriocin- producing LAB and HPT, count of *S. aureus* reached 6.67 log CFU g^{-1} but with the inoculation of bacteriocin with HPT the count of *S. aureus* is gradually decreasing. Some uses of bacteriocins with hurdle technology listed in the table 4. Another examples of hurdle technology is that nisin in combination with the pulse electric field (PEF), which increases the permeability of cell membrane, can also act at the level of the cell wall. [99] Nisin is generally less effective against gram negative bacteria but the growth of these bacterial like *E.coli* O157:H7 and *Salmonella* can be controlled when EDTA, a metal chelator is used in combination with nisin. It disrupts the outer membrane which allow penetration of nisin. [100]

For more than three decades, *Listeria monocytogenes* continuously affecting food safety especially in ready-to-eat food where it cause listeriosis. [109] The lyophilized culture of *Lactococcus lactis* LMG21206 and *Lactobacillus curvatus* LBPE are directly

incorporated in the sausage fermentation to control the effect of *Listeria monocytogenes*. The fermentation was carried out at pH 6.0 with moderate agitation of 100 rev/min. The results shows that the bacteriocins produced by *Lactococcus lactis* LMG21206 and *Lactobacillus curvatus* LBPE were effective against *Listeria* strains tested individually in dry sausage fermentation. In cheese production the spoilage due to *Clostridium tyrobutyrium* is controlled by the use of bacteriocin producing *Enterococcus* as starter culture. [110-111] Bacteriocin producing Enterococci or purified enterocins have been used as starter culture or protective culture in the production of various food products. [112] Microcin a bacteriocin is a 21- residual ribosomally synthesised lariant peptide antimicrobial compound, which is active against food borne pathogens like *Salmonella, Shigella and E.coli*. To observe the antimicrobial activity of microcin MJ25 , sterile skim milk and diluted egg yolk were inoculated with approx 10^4 cells/ml of *E.coli* O157:H7 in the presence and absence of microcin and incubated at 37˚C. After incubation, the food sample were observed. Microcins effectively inhibit the growth of pathogenic strains in milk and egg yolk. [113] One more bacteriocin isolated from *Pediococcus acidolactici,* KP10 is a potential antimicrobial compound that may show beneficial effect on intestinal microflora because the strain is tolerant of bile salts(0.3%) and acidic conditions (pH 3). [114]

Bacteriocins from *Bacillus* have a large number of potential food application like in dairy products such as milk and cheese. Two examples are bacillocin 490 and cerein 8A. Bacillocin 490 is active against *bacillus spp*. both in aerobic and anaerobic conditions. Also they were tested to control the growth of *Listeria monocytogenes*. The addition of 160 AU ml^{-1} of cerein 8A in milk resulted in a decrease of 3 log cycle in viable cell with in 14 day period at 4˚C. [115] When cheese is treated with cerein 8A , it showed a 2 log cycle decrease in viable cell during 30 days at 4˚C to control the growth of *Listeria monocytogenes.* Lactobacillus curvatus MBsa2 and MBsa3 isolated from italian salami samples produce two bacteriocins sakacin P and sakacin X with high stability of pH, heat and Nacl and they are very effective against *listeria* starins when these bacteriocins are used in semi-purified form in the batter for production of salami. It cause 2 log CFU/g count reduction in the final product when compared to salami without the addition of bacteriocin. This shows the safety of ready-to-eat food in relation to *listeria* starins. [116] Some suggested applications of bacteriocins are discussed in table 5.

Many of the bacteriocins produced by *bacillli* inhibit plant-producing strains could be applied

in the biological control of plant disease. One bacteriocin ericin S produced by *bacillus spp*. is active against *C. michiganesis* which is the causative agent of tomato bacterial canker. [127] Another bacteriocin Bac 14B produced by *Bacillus subtilis* 14B isolated from rhizosphere is potentially applied as a biocontrol agent for reducing *Agrobacterium tumefaciens* infection caused in plants.

Cancer therapy

Cancer has become a threat to human health and a most serious problem over past half century. According to WHO, 8.2 million people died from cancer. In 2013 there were 1,660,290 new cancer cases with 580,350 cancer deaths projected in the United States. Due to this cancer became the second leading cause of death. [128] Some researchers indicated that bacteriocins are effective against tumor cells. bacteriocins are natural food additives that are suitable as a potential anti-tumor drug. There are some bacteriocins like colicin E1 and A having pore forming mode of action, inhibited the growth of human fibroblast line MRC5 and 11 human tumor-cell lines. [129] In the faeces of 160 healthy people 63.8% people had bacteriocin producing *E.coli* which showed that colicin from *E.coli* in the intestine is one of the factor in reducing human colon-rectal cancer. These *E.coli* producing colicins acts as anti-cancer drug of moderate potential. In a study by Joe et al., 2014 found that nisin had capabilities to prevent cancer cell growth. Three cancer cell 17B, 14A, and HSA were treated by nisin at concentration of 40 and 80 µg/ml. After 24 hours nisin increased DNA fragmentation and reduced cell proliferation of HNSCC occurred. In this study the floor-of-mouth oral cancer xenograft mouse model was used to test the anti-HNSCC function of nisin. These studies showed that nisin provide a safe and novel therapy for treating HNSCC.

CONCLUSION

Quality packaged food is the prime demand of ever growing population at global level and similarly wants to reduce dependency on chemical food preservatives since it cause serious health problems. Beside antibiotics some microorganisms are identified who are producing short cross-linked peptide "Bacteriocin" which has excellent antimicrobial properties. Bacteriocin producing microorganisms are generally found where complex microbial diversity develops besides extreme fluctuation in biotic and abiotic environment like large intestine of human and animals, fermented dairy products, rhizosphere, aerobic-anaerobic liquid waste treatment plant and biofilms. Since discovery it was globally accepted by various food industries as alternative of chemical preservatives by

developed countries and its market is continuously growing. Food & Beverage industries are one of the highly unorganised sector in developing countries, it is an opportunity because of market size and increasing demand of packaged food. Bacteriocins producing microorganisms from various sources are safe for human consumption and which are discussed in Table 2. Production of these compounds and its uses in food industries is validated by several researchers. Some researchers in recent past also identified that bacteriocins are helping gut microflora and balance the microbial ecosystem and human health besides working against pathogenic microorganisms. It has open new avenue for microbiologists to search more and more microorganisms who have extra capacity of bacteriocin production. Similarly it is a challenge for molecular biologists to improve the existing strains and developed genetically engineered for higher production. Bacteriocins will be in high demand in next decade for its antipathogenic microorganism properties as well as its great contribution in balancing healthy microbial ecosystem in gut which is directly linked with human health.

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Table 1:- Chemical Preservatives and their harmful effects

Chemical Preservative	Applicable range mg/kg body weight	Industrial Use	Health Effects
BENZOATES	0-5	Prevent form yeast & moulds	Hyperactivity; Dizziness, light-headedness, trouble sleeping; DNA damage, Parkinson and ageing. In combination with ascorbic acid (vitamin C, E300), it may form benzene, a known carcinogen
NITRITES	0-7	Antioxidants & antibacterial	Irritability, headaches, pregnancy complications, infact health problems, Leukaemia, colon, bladder cancer
SULFITIES	0-0.7	Antibacterial	Hay fever; runny nose, itchy eyes and wheezing cough. 1 in 10 people will have a reaction of some sort eg. Rashes, itching, restricted breathing, asthmatic attacks, hives, cramps.
SORBATES	3	Prevent form yeast	Hypersensitivity (reaction, allergy) to mouth, throat, eyes. Migraine/ headache
ASPARTAME	40	Antibacterial	Headache, alter level of serotonin and cause behavioural problems, cancer
PROPIONIC ACID	10	Antibacterial	neurobiological effects characteristic of autism spectrum
TERTIARY BUTYLHYDROQUINONE (TBHQ)	0.02	Antioxidant	Consuming high amounts of the preservative can cause nausea, vomiting, dizziness, and hyperactivity in children

Table 2:- Different Sources for isolation of bacteriocin producing microorganisms

Sources	Isolated Strain	Active against	References
Vegetables	*Enterococcus mundtii*	*L. monocytogenes*, *C. botulinum*	[51]
Bean-sprouts	*Lactococcus lactis*	*L. monocytogenes* Scott A	[52]
Spoiled ham	*Clostridium piscicola* JG126	*L. monocytogenes*	[53]
Dry sausage	*Lac. plantarum* UG1	*L. monocytogenes*, *Bacillus cereus*, *C. perfringes*, *C. sporogenes*	[54]
Dry fermented sausage	*Lac. lactis (NisA)*	*L. monocytogenes*	[55]
Red smear cheese	*Brevibacterium lines* M18	*Listeria and corinebacterium spp.*	[56]
Sauerkraut	*Lac. lactis* subsp. lactis ŽNis.	*L. monocytogenes*	[57]
Whey	*Ent. faecalis* 226	L. monocytogenes	[58]
Munster Cheese	*Lactob. plantarum* WHE92 ŽPedAcH.	L. monocytogenes	[59]
Traditional French cheese	*Ent. faecalis* EFS2	*L. inocua*	[60]
French mold-ripened soft cheese	*Carnobacterium piscicola* CP5	*Carnobacterium*, *Listeria*, and *Enterococcus* spp.	[61]
Radish	*Lac. lactis* supsp. *cremoris* R	*Clostridium*, *Staphylococcus*, *Listeria*, and *Leuconostoc* spp.	[62]
Bulgarian yellow cheese	*Lactob. delbrueckii* sp.	*L. monocytogenes*, *Ent. faecalis*, *E. coli*, *Yersinia enterocolitica*, *Y. pseudotuberculosis S. aureus*,	[63]
Irish kefir grain	*Lac. lactis* DPC3147	*Enterococcus*, *Clostridium*, *Listeria*, *Leuconostoc* spp.	[64]
Commercial probiotic product	*Streptococcus* sp. CNCM I-841	*L. monocytogenes*, *Clostridium* sp.	[65]
Goat's milk	*Leu. mesenteroides* Y105	*L. monocytogenes*	[57]

Table 3:- Countries in which nisin is using [97]

Country	Permitted food	Maximum range (IU/g)
US	Processed cheese	10,000
Mexico	Use as additive	500
France	Processed cheese	No limit
UK	Cheese, canned food	No limit
Peru	Use as additive	No limit
EU	E234 labelled as natural preservative	Varies according to product
Russia	Canned vegetables,	8000
Argentina	Processed cheese	500
Cyprus	Clotted cheese, cheese, canned vegetables	No limit
Australia	Canned tomato	No limit
Netherland	Factory cheese, cheese powder	800
Belgium	Cheese	100
Italy	Cheese	500

Table 4:- Use of bacteriocins in hurdle technology

Bacteriocin	Inactivation effect	References
Nisin	When used with EDTA, lactate, citrate, it is effective against gram negative bacteria like *salmonella, Trphimurium and E.coli* O157:H7	[101]
Pediocin AcH	When used with combination of temperature and pressure it cause reduction of viability of *S. aureus, L. monocytogenes, E.coli* O157:H7	[102]
Nisin	When used with modified atmosphere packaging and low temperature, it increases the lag phase of *L. monocytogenes* and prevents its growth	[103]
Pediocin AcH	With emulsifier (tween 80), it show higher listericidal activity in milk, buttermilk and meat	[104]
Nisin	With pulsed electric field, it show synergistic activity against *B. cereus*	[105]
Nisin	In combination with heat, it enhances inactivation of *L. monocytogenes* and *Salmonella* by mild heat	[106]
Curvaticin	In addition with nisin it induces inhibitory effect against *L. monocytogenes* than the use of single bacteriocin	[107]
Leucocin F10	In combination with nisin, leucocin F10 gives greater activity against *L. monocytogenes*	[108]

Table 5:- Some suggested applications of bacteriocins

Bacteriocin	Application	References
Nisin A	Use of nisin to control L. monocytogenes in ricotta cheese	[117]
Enterocin	Add enterocin to inoculated ham, pork, chicken breast, pate, sausage	[112]
Nisin A	Incorporation of nisin into a meat binding system	[118]
Pediocin	Expression of pediocin operon in *Sac. cerevisiae*	[119]
Lactocin 705	Use of lactocin 705 to reduce growth of *L. monocytogenes* in ground beef	[120]
Pediocin PA-1	Use of *P. acidilactici* strain as a starter culture in sausage fermentation	[121]
Leucocin A	Use of a leucocine-producing *Leu. gelidum* UAL187 to control meat spoilage	[122]
Pediocin AcH	Use of a pediocin AcH producer *Lactob. plantarum* WHE 92 to spray on the Munster cheese surface at the beginning of the ripening period	[59]
Linocin M-18	Use of *Bre. lines* as a starter culture for production of red smear cheese	[123]
Pediocin AcH	Add pediocin preparation to raw chicken	[124]
Pediocin AcH	Use of the pediocin producer *P. acidilactici* to inhibit *L. monocytogenes*	[125]
Piscicolin 126	Use of piscicolin 126 to control *L. monocytogenes* in devilled ham paste	[53]
Enterocin 4	Use of an enterocin producer *Ent. faecalis* INIA4 as a starter culture for production of Manchego cheese	[126]