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Bacteriocins As Next-Generation Antimicrobials In Food Preservation

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Abstract: Bacteriocins are considered a blessing for the food industry. They act as a preservative to inhibit the growth of bacteria in the food items during the production or preservation process. These bacteriocins are produced by the bacteria, which reduces the growth of closely related bacterial strains. Food safety has become a serious issue in the present era for which bacteriocins, an antimicrobial compound, can effectively fight against food-pathogenic bacteria. Different views of the actions of bacteriocins have been elaborated. Bioengineered bacteriocins, or the hurdle approach to bacteriocins, can increase the efficacy of preservation and reduce the resistance effect against bacteriocins. They are mostly produced by LAB (Lactic acid bacteria), which is naturally present in most food. They can also be combined with antimicrobial proteins, agents, and natural phenolic compounds. The combined approach can act as a barrier to developing bacteriocin-resistant strains. It can also be combined with physical treatments like pulse electric fields and high-pressure processing by which foods can be more effectively preserved. This chapter summarizes and focuses on the importance and potential applications of bacteriocins as a food preservative in the food industry.

Keywords- Bacteriocins, Bio preservative, Lactic acid bacteria, Hurdle technology

1. INTRODUCTION

Food preservation is still a big question mark in this modern era of technology in both developed and developing countries. The food spoilage caused by food-borne pathogenic bacteria is a major challenge in the food industry. Food is a very delicate and important part of our everyday routine. Preventive measures should be taken for its safety and stability before consumption. Food products are preserved to maintain nutritional, physicochemical, and functional qualities. For long-term preservation, chemical preservatives restrict the growth of some pathogenic bacteria isolated from food. Long-term intake of these synthetic preservatives could kill the gut microflora, i.e. both healthy and pathogenic bacteria, which leads to several diseases like breathing issues, cancer, obesity, etc. (Bharti et al., 2015). Due to the food safety standards and the need for customers to use free/natural preservatives in foods, classical preservation techniques and preservatives are discouraged. Because of the proven adverse effects of synthetic preservatives and classical preservation techniques, several allergic reactions and carcinogenic end products have been observed (Kashani et al., 2012). The above-stated problem attracted the researchers to find an alternative preservation process. Due to the rise in spoilage issues, many artificial preservatives are coming forward, but consumers still prefer safe and natural preservatives, i.e. "The Biopreservatives". The preservation technique, including the microorganisms or their natural products, is termed "Biopreservation" (Kumariya et al., 2019). In recent days, the use of non-pathogenic bacteria and their by-products for food preservation techniques is gaining more attention. The application of biopreservation strategies in the food industry is creating high demand. One of these biopreservation strategies is using bacteriocins as a natural preservative in food industries.

The antimicrobial proteins (AMPs) synthesized by the ribosomes are called "Bacteriocins". The ribosomally produced bacteriocins are proven to have antimicrobial activity at a specific concentration (Chikindas et al., 2018). Proving their biotechnological importance, the bacteriocins have antimicrobial potential against the pathogenic food-borne bacteria. The first reported Bacteriocin, i.e. Colicins, was derived from *E. coli* in 1925 (Gratia, 1925). Bacteriocins produced from Lactic Acid Bacteria (LAB) have gained particular importance from the American Food and Drug Administration (FDA). One of the most common Bacteriocin, i.e. Nisin developed from *Lactobacillus lactis* is considered to be the first Bacteriocin to gain worldwide commercial acceptance (Zacharof and Lovitt, 2012). The LAB bacteriocins have received widespread importance because of their heat stability properties and wide pH range. These bacteriocins are considered to be tasteless, colourless and odourless. They are proteinaceous, so they easily degrade the proteolytic enzymes (Perez et al., 2014). The bacteriocins target a specific bacterial pathogen and are considered designer drugs. Bacteriocins are produced by both Gram-positive and Gram-negative bacteria, i.e. *Enterobacteriaceae* family and Lactic acid bacteria, *Bacillus* species, etc. (Prabhakar et al., 2013).

1.1 Chemical Preservatives: A silent toxin

Chemical preservatives are substances which cause health hazards. Most chemical preservatives are considered safe up to a certain limit. But the fact that cannot be ignored is that they have numerous negative and life-threatening side effects. Table 1 indicates some health hazards caused by certain commonly used preservatives. Certain food additives or preservatives show allergic reactions when taken orally immediately or in a few days. The compound mixes with the blood and shows allergic reactions. Sometimes, finding the exact cause of allergic reactions is difficult. So it is imperative to avoid chemical preservatives mixed food products and consume preservative-free products or food products prepared by biopreservation techniques (Anand and Sati, 2013).

1.2 Classification of Bacteriocins

1.2.1 Classification of Gram-positive bacteriocins

Based on the chemical structure, physical properties, molecular size, stability, and mode of action, bacteriocins are classified into four groups (Figure: 1). Various debates have been conducted to classify bacteriocins in gram-positive bacteria. This chapter incorporates the classification according to Zimina et al., 2020. The Gram-positive bacteriocins are further classified into the following four classes:

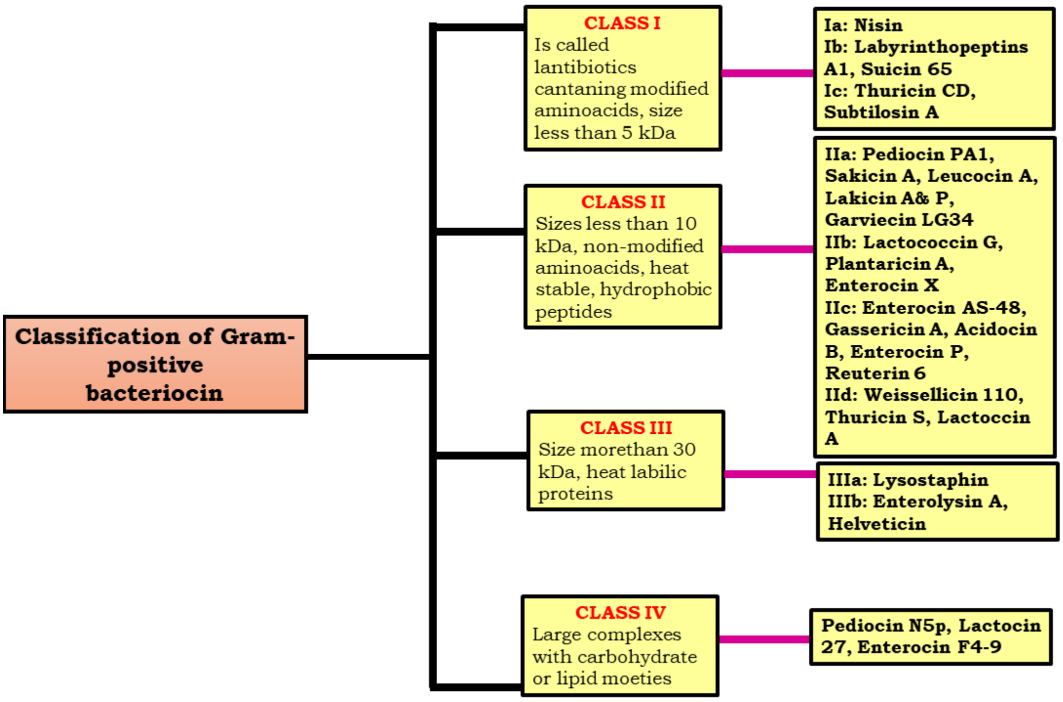

Figure: 1 Classification of Gram-Positive Bacteriocins

Table 1: Health risks of some commonly used chemical preservatives

Chemical Preservatives	Food Containing	Health Risks	References
Butylated Hydroxytoluene (BHT)	Packet Cake Mix, Dehydrated Mashed Potato, Potato Crisps, Salted Peanuts	Liver, Thyroid and Kidney Problems	Anand and Sati, 2013
Butylated Hydroxytanisole (BHA)	Baked Goods, Beer, Butter, Cereal, Chewing Gum, Dessert Mixes	Skin Allergy	Bharti et al., 2015
Tetra Butylhydroquinone (TBHQ)	Vegetable Oils, Food Packed Products, Margarine, Snacks, Instant Noodles, Bakery Products	Hypersensitivity, Asthama and Cancer	Anand and Sati, 2013
Potassium Sorbates	Cheese, Yogurt, Apple Cider, Soft Drinks, Dehydrated Fruits, Wine	Urticaria And Contact Dermatitis	Sharma, 2015
Calcium propionates	Bread, Whey, Processed Meat, Baked Goods, Dairy Products	Stomach Cancer	Sharma, 2015
Sodium nitrate	Cured Meat, Sausage, Ham, Bacon, Smoked Fish	Hypersensitivity, Asthama and Cancer	Anand and Sati, 2013
Benzoic acid	Fruit Products, Acidic Foods, Margarine	Allergies, Asthama and Skin Rashes	Sharma, 2015
Sodium benzoate	Carbonated Drinks, Margarines, Tomato Paste, Sauces, Fruit Juices, Mayonnaises	Presence Of Neurotoxic and Carcinogenic Compounds Which Leads to Fetal Abnormalities, Aggravates Asthama	Yadav and Gupta, 2021
Sulphur dioxide	Sausages, Vinegar, Wine, Cider, Pickled Vegetables, Dried Fruits, Fruit and Vegetable Juices	Gastric Irritation, Diarrhea, Asthama Attacks, Nausea, Skin Rashes	Yadav and Gupta, 2021
Propyl Gallate	Cereals, Snack Foods, Pastries	Allergy, Asthama and Cancer	Anand and Sati,2013
Hexamethylene Tetramine	Fish And Marinated Muscles	Presence Of Carcinogenic Compounds	Anand and Sati, 2013
Sodium metabisulphite	Maple Syrup, Jam, Jelly, Bread, Pizza Dough, Biscuits	Breathing Problem, Lungs Irritation, Asthama Attacks	Anand and Sati, 2013
Propylparaben	Pancake Syrup, Muffins, Wine, Canned Food Products	Estrogen Related Risks, Breast Cancer, Asthama	Golden et al., 2005
Potassium bisulphite	Fruit Juices, Squash, Chutney	Hypersenstivity, Asthama	Anand and Sati, 2013
Aspartame	Tabletop Sweetener, Beverages, Drink Mixes, Chewing Gums	Neurological Damage	Yadav and Gupta, 2021

Class I type

Due to the existence of some unusual amino acids (dehydroalanine, dehydrobutyrine, lanthionine, etc.), the post-translationally modified peptides are termed "Lantibiotics". They contain 19-50 amino acids (McAuliffe et al., 2001). The size of these peptides is <5 kDa. Due to the presence of lanthionines, Lantibiotivs are thermostable (Balciunas et al., 2013). The thermal stability is due to the post-translationally modified pro-peptide region, especially in the residues of threonine, serine, and cysteine (Brotz et al., 1998).

Class II type

These are translationally unmodified small peptides with a size of <10 kDa. These are further divided into three subgroups: Class IIa: These are thermostable due to the absence of lanthionine and its derivatives. Pediocine-like peptides in the N-terminal have a mass of 3-7 kDa and a chain length of 25-58 amino acids (Fimland et al., 2000). **Class IIb:** These multicomponent bacteriocins require adding unmodified peptides to provoke antimicrobial activity. Enterocin 1071 (A & B) obtained from *Eenterococcus faecalis* BFE1071 is a example (Nissen-Meyer et al., 2010). **Class IIc:** These single peptides have a molecular mass of 3.5-7.5 kDa. They have different sites of action for targeting the susceptible strains. Example: Enterocin Ej97 (Johnson et al., 2017).

Class III type

These are bulky proteinaceous peptides consuming a molecular mass of >30 kDa. They are commonly known as bacteriolysis, which causes the cell wall lysis of the specific strains through enzymatic reaction. They have been further subdivided into two sub-groups: Class IIIa and b **(**Johnson et al., 2017).

Class IV type

These are the unique circular bacteriocins having linear molecules. The molecular weight varies from 5.5 to 7.5 kDa and the chain length ranges from 30 to 75 amino acids. It shows antimicrobial activity due to its hydrophobic properties (Sanchez-Barrena et al., 2003). Example: Enterocin AS-48. The bacteriocins available in the markets, like Nisin, pediocin, enterocin, etc., are widely used for food preservation because of their action over a wide range of pH and heat stability.

1.2.2 Classification of Gram-negative Bacteriocin:

They are developed from gram-negative bacteria are categorized according to their size as described in Figure: 2. Further, these bacteriocins are classified into four sub-classes: Colicins, Colicin-like, Phage-tail-like and Microcins (Zimina et al., 2020). Colicins: It has a molecular weight of 30-80 kDa. These are mostly heat-sensitive protease sensitive. These are obtained from the *E. coli* strains having an only colicinogenic plasmid under stress conditions. (Sun et al., 2018). Colicin-like: These also have alike structural and functional features. The receptor binding takes place in the central R domain, and the translocation occurs in the N-terminal T-domain, which allows the penetration of the bacteriocin into the cell. The active centre of the Bacteriocin is the C-terminal C-domain, which causes cytotoxicity (Tracanna et al., 2017). Tailocins: The molecular mass consistes of 20-100 kDa. It comprises 8-14 peptide subunits, similar to the bacteriophage tail molecules. Clustered genes in the genomes of bacteria larger than 40 kbp are encoded for the tailocins (Tracanna et al., 2017). Microcins: It has a molecular mass of <10 kDa. These peptides are highly stable and responsible for the Enterobacteriaceae family's interactions. They can tolerate a wide pH range, protease enzymes and temperature values (Ongey et al., 2017).

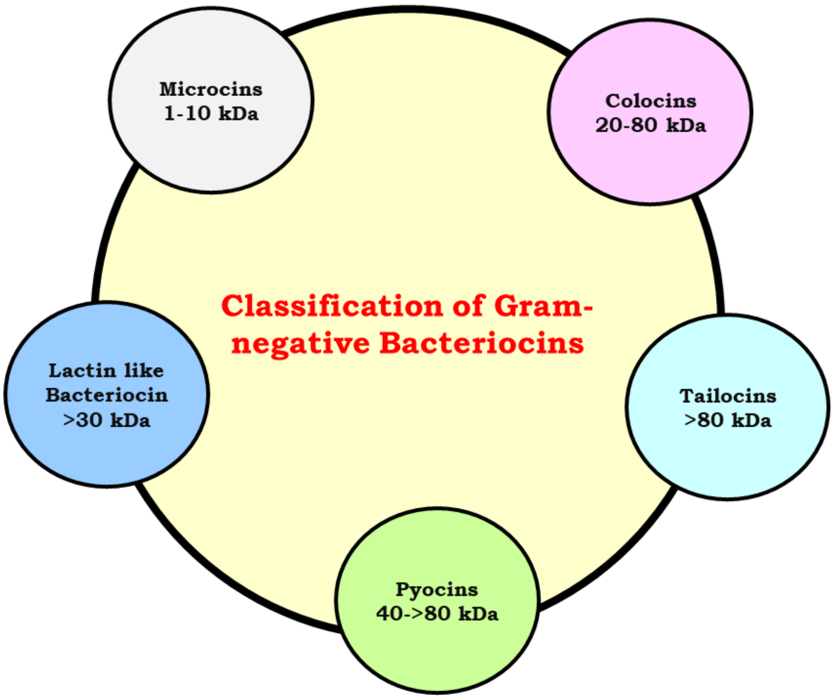

Figure: 2 Classification of Gram-Negative Bacteriocins

1.3 Synthesis of Bacteriocins

The genetic factor responsible for the bacteriocins' production are generally present in the operon bunches, sheltered in the genome, plasmids and other mobile genetic rudiments. For induction, the auto-inducer peptide is required, and the communication of operons is inducible (Uzelac et al., 2015). The expression of these operons is regulated by a component regulatory system (Eijsink et al., 2002; Kleerebezem et al., 2001). Precursors usually synthesize the bacteriocins, which are further processed and possibly transformed (Morton et al., 2015). Further, the cleavage of the bacteriocins leads to the generation of the mature form. The genes being further modified and exported are close to those responsible for bacteriocin biosynthesis. The Bacteriocin secretion uses carriers like ABC transporters and sec-dependent exporters (Chatterjee & Raichaudhuri, 2017). The cationic Bacteriocin target the bacterial cell surface, which comprises cardiolipin, phosphatidylethanolamine, lipopolysaccharide, and phosphatidylglycerol (18). They change their configurations for proper interactions. Some bacteriocins require target receptors (lipid II or mannose permease) for docking, while some of them do not require receptors for docking (Hechard & Sahl, 2002).

2. MECHANISM OF ACTION

2.1 Mode of action

There is various mode of actions of bacteriocins which varies from both the gram-positive and gram-negative bacteria. They mainly disrupt the cell wall and inhibit protein and cell wall synthesis (Cascales et al., 2007). They mostly target the cell wall components, which enables cell lysis, which results in cell death (Chikindas et al., 1993). In Gram-negative bacteria, the cytoplasmic membrane is attacked by Nisin with the combination of ethylene diamine tetra-acetic acid (EDTA). Likewise, in Gram-positive bacteria, mersacidin inhibits cell wall synthesis (Bottiger et al., 2009). Other bacteriocins cause cell death and help preserve food (Table- 2).

Table- 2: Mechanism of action of bacteriocins against Gram positive and Gram-negative bacteria

Bacteriocins	Producing Bacteria	Mechanism of action	References
Lysostaphin	*S.aureus*	Cell wall lysis	Grundling & Schneewind, 2006
Colicin A	Gram negative bacteria	Inhibits the biosynthesis of protein	Cascales et al., 2007
Pesticin	*Yesinia spp. & E. coli*	Cell wall degradation	Cascales et al., 2007
Lacticin	*Listeria*	Inhibiting cell wall synthesis and by pore formation	Cascales et al., 2007
LtnA1	Gram negative bacteria	Cell wall lysis	Kajos et al., 2009
Antimicrobial peptides	Gram positive bacteria & Gram-negative bacteria	Inhibits DNA/RNA/Protein or cell wall synthesis	Abee et al., 1994

2.2 Approaches to mitigate bacteriocin resistance

Bacteria develop several resistance mechanisms to resist the bacteriocins (Gradisteanu Pircalabioru et al., 2021). So, bacteriocin alone cannot control the bacteria responsible for food spoilage. The dosage of Bacteriocin causes several side effects, and sometimes, it might be harmful to consumers (Tang et al., 2022). Pairing of antimicrobial agents with the bacteriocins is considerable. Several plant-derived phytochemical constituents have some promising results (Patel, 2015).

Several studies have shown the significance of using two bacteriocins such as pediocin AcH and Nisin (Hanlin et al., 1993), leucocin F10 paired with Nisin (Parente et al., 1998), pediocin AcH paired with lacticin 481 (Mullet-Powell et al., 1998).

2.3 Bacteriocins- an alternative to antibiotics

There is an urgent need for innovative solutions to address the significant growth of infections caused by antibiotic-resistant bacteria. The antimicrobial peptides, i.e., 'Bacteriocins' produced by certain group of bacteria, might be considered an alternative to the antibiotics. These bacteriocins are stable and can have broad or narrow spectra ranges. They can exhibit a potential activity against pathogenic bacteria or antibiotic-resistant bacteria. Several known bacteriocins' properties prove they can be a potent substitute for antibiotics (Cotter et al., 2013).

The significant role of bacteriocins against pathogenic bacteria varies within different peptide sub-classes. Thiopeptides and lantibiotics belong to class I bacteriocins and are effective against Gram-positive pathogenic bacteria. Lantibiotics, such as planosporicin, Nisin, epidermin, Pep5, and galledermin, are active against the most common Gram-positive bacteria like *Streptococcus pneumonia*, MRSA, *Clostridium difficile*, VRE (Piper et al., 2009). Thiopeptides are also active against Gram-positive antibiotics, but commercial production is impossible due to their poor stability (Zhang et al., 2009).

The efficacy of the single bacteriocins could be further boosted by combining the bacteriocins with certain traditional or new-generation antibiotics to increase their potential against the pathogenic Gram-positive and Gram-negative bacteria. When combined with polymyxin E and clarithromycin, an antibiotic, Nisin exhibited synergistic activity against *Pseudomonas aeruginosa* (Giacometti et al., 2000).

3. BACTERIOCINS: AS BIOPRESERVATIVES

3.1 Common Bacteriocins

Listed below are some of the bacteriocins that are commonly used as food preservatives: Enterocins**:** Enterocins are generally formed by *Enterococci* species, which fit to the group of Lactic acid bacteria and are isolated from various food products such as cheese, fish, meat, sausages, etc. These bacteriocins have bactericidal activities against *Staphylococcus aureus, Listeria monocytogenes, E. coli, Clostridium* sp., *Bacillus cereus, Vibrio cholera*, etc. (Elsilk et al., 2015). Pediocins: They belong to the class II unmodified antimicrobial peptides. They have antibacterial properties, or they are active against the *Listeria* sp. This group of bacteriocins are produced by *Pediococcus* sp. The medicines are constant under the composite environment of the food. Pediocin F is formed from *P. acidilactici* and is mostly isolated from fermented sausages. (Loessner et al., 2003). Acidocin: Plasmid encoded acidocin B is produced from *Lactobacillus acidophilus.* Bacteriocin has shown a wide range of inhibitory activity against *Clostridium sporogenes, L. fermentum, Brochothrixthermo sphacta, and Listeria monocytogenes* (Leer et al., 1995). Mesentericin Y105: The bacteriocin is produced by *Leuconomostac mesenteroides.* It has displayed it's *in vitro* activity at a very low concentration, i.e. 2 µg/ml activity against *E. faecalis* and *Listeria monocytogenes* (Morisset et al., 2004). Curvacin A is a small peptide of 38-41 amino acid residues. In the late exponential growth phase, the *Lactobacillus curvatus* produced Curvacin A. The Bacteriocin is active against food-borne pathogens like *L. monocytogenes*, *Enterococcus faecalis* (Ahsan et al., 2022).

3.2 Bacteriocin in the food system

As bacteriocin acts as an antimicrobial agent, it has numerous functions in the food system. It works against food-borne pathogenic bacteria and helps preserve food against spoilage microorganisms. As already discussed, Nisin is a common bacteriocin used to control the significant growth of microorganisms. Nisin can prevent the development of bacterial spores after germination, and it is also helpful in maintaining the *Clostridium botulinum* in processed cheese foods (Hassan et al., 2021). All the commercially available bacteriocin work in two different ways: (a) by directly adding the Bacteriocin in the food products and (b) by bacteriocin-producing bacteria as a starter culture. Many lactic acid bacteria (LAB) are added to food products intentionally to enhance the preservation process and to get the desirable commercial products, i.e. pickles, sausages, yoghurt, etc.

Several factors affect the efficacy of the bacteriocins in food, including the type of food and type of bacteriocins used to preserve or inhibit the growth of food-borne pathogens. For example, Nisin is mostly effective in the acidic pH, low protein and low fat content food and at pH 2.0 nisin is heat stable and mostly soluble. The solubility and heat stability decreases with the increase in pH (Peng et al., 2020).

3.3 Factors affecting the usage of Bacteriocin as bio-preservatives

Several factors affect the stability and efficacy of bacteriocins as bio-preservatives in the food systems. The factors include water activity, fluctuation in the temperature, unfavorable pH, freezing, thawing, heating, etc. The efficacy of the bacteriocins is also affected by adding spices, additives, salt, texture, buffering capacity etc. Resistant to the pathogenic strains, genetic instability also reduces the efficacy of the bacteriocins. The factors mentioned above correlate and affect the bio-preservation technique (Galvez et al., 2007). The effectiveness of the bacteriocins is directly dependent upon the permeability of the bacteriocins in the food and the pH. The pH alters the bacteriocins' solubility**.**

4. BACTERIOCINS AND ITS USES

4.1 Bulk Production of Bacteriocins

The process includes biological systems to produce bacteriocins in a huge amount at a very low or economical cost. Several factors are included for the bulk production of the bacteriocins, including inocula size, composition of the desired medium (minerals, nitrogen & carbon), pH, temperature, inducers, etc. According to the reports, the demand for bacteriocins is rising daily. The present market value (2022) of bacteriocins is USD 424.65 million and is expected to be USD 663.38 million by 2029. The major consumers of bacteriocins are North America, Europe, Asia Pacific, Middle East and Africa, and South America (Figure: 3). The major key players in this group include CHR Hansen, DowDuPont, Sacco S.R.L, CSK food Enrichment, B.V, THT S.A, Dalton Biotechnologies, etc.

4.1.1 Inoculum size

Inoculum size shows a significant role in the manufacture of bacteriocin. Ideal inocula size is advantageous for the maximum output of the Bacteriocin. 1% (1 x 10^7 CFU/mL) of *B. cerus* 8A was inoculated in brain heart infusion (BHI) medium at 30^0 C into 100ml production medium (Dominguez et al., 2007). Likewise, 1% inocula of *B.subtilis* EMD4, *B.velezensis* BS2 was cultured in LB broth before the addition of starter culture into the bacteriocin producing media (Kindoli et al., 2012). More is the inocula size short is the fermentation time. However, adding more inocula can consume more nutrients, ultimately increasing the production cost. The inocula size may vary from strain to strain due to medium size, cell proliferation rate, mass transfers, etc. Low inocula size may affect the production of bacteriocins. The greater the inoculum size, the faster the growth of bacteria, and the more Bacteriocin is produced. Hence, the size of the inoculum affects the production of bacteriocins. It significantly prepares the starter culture, which involves the Bacteriocin's productivity (Huang et al., 2016).

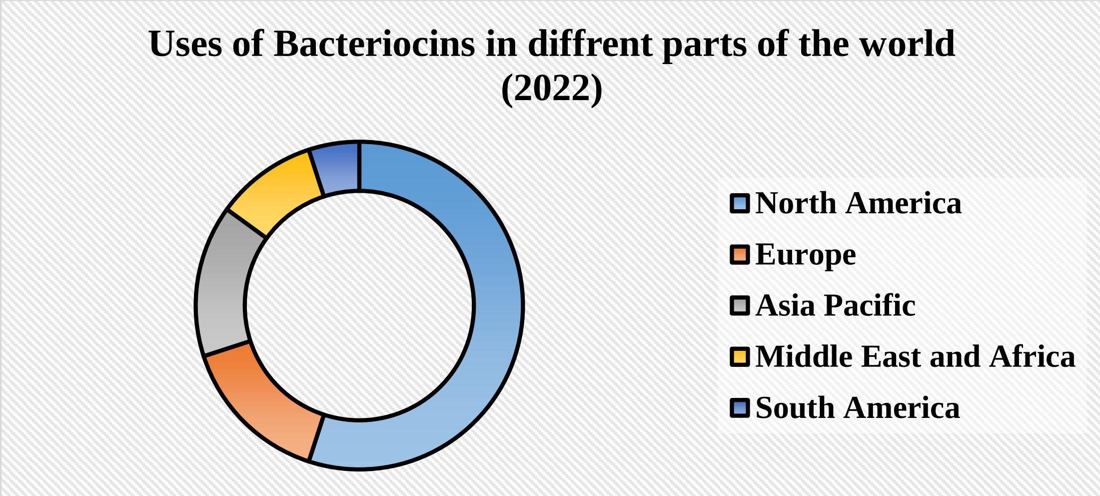

Figure: 3 Uses of Bacteriocins in different parts of the world (2022)

4.1.2 Composition of the desired medium

Growth medium plays a significant role in the development and expression of the bacteriocins, which also vary from species to species. Enzymes such as oxidoreductase, lanthipeptide, etc., are affected by the composition of the desired medium for the production of the Bacteriocin. Apart from necessary enzymes, certain amino acids are also required to synthesize the bacteriocins. The media used to produce bacteriocins are MRS, BHI, etc., a rich source of vitamins, minerals, nutrients, carbon, etc. MRS, BHI & M17 media are used to produce bacteriocins on an industrial scale from the strains of *B. subtilis* NS02, *Bacillus* sp. SW1-1, *B. subtilis* R75 etc. (Kim et al., 2014). The yield of Bacteriocin EMD4 was highest when grown in BHI medium compared to TSB, NB, lactose broth (Saleem et al., 2009).

The presence and absence of minerals are also responsible for the production of the bacteriocins. The low concentration of KNO_3 and $K_2 HPO_4$ has shown a positive effect on cell growth and leads to the bulk production of bacteriocins MKU3. Due to the osmotic stress of the NaCl (2.5%), there is an increase in the production of bacteriocins MKU3. Different minerals are important in producing elgicins, like $CaCl_2$, $K_2 HPO_4$, $MgSO_4$, NaCl, $ZnSO_4.7H_2O$, etc. (Teng et al., 2012). Metal ions such as Mg^{2+}, Fe^{3+}, and Mn^{2+} are also responsible for the production of the bacteriocins, but above 0.5%, these may show negative results (Teng et al., 2012).

4.1.3 pH and temperature

The pH plays a vital role in the production of Bacteriocin. Initially, the pH supports the growth of the bacterial strains. And when the desired pH is obtained, it favours the bacteriocin production. The maximum output of the bacteriocin BLIS by *B. licheniformis* was obtained at pH 6.5-7.5. At an initial pH of 6.5-9.0, Cerein 8A was synthesized in huge quantities (Dominguez et al., 2007). At pH 7.0 and 30 °C, bacteriocin SW-1 showed highest production (Kim et al., 2014).

For the manufacture of Bacteriocin, a temperature range of 29-40 °C is required, but above 40 °C it is considered unfavourable (Patrick et al., 2008). When the temperature is maintained at an optimum level, there is a quick cell propagation, which improves the synthesis of important enzymes like oxidoreductase, lanthipeptide peptidase, which helps produce the bacteriocins (Patrick et al., 2008). The maximum yield of the various bacteriocins was obtained at different temperature ranges; BLIS grows at 30 0C, Cerein 8A grows at 22 and 34 ^{0}C (Huang et al., 2016).

4.1.4 Inducers

Bacteriocins act as an inducer for its biosynthesis (Schmitz et al., 2006). Two-element Spa RK in *B. subtilis* activates the subtilin, which also contains 32 amino acids (Geiger et al., 2017). When sporulation occurs in 72 and 96 h, the bacteria *B. subtilis* itself produce the bacteriocins due to unfavourable conditions and differences in the media composition (Garg et al., 2014). Sawdust, thermocol beads, and straw powders could also be added to increase the production of lichenin produced by *B. licheniformis* by 35% (Garg et al., 2014).

4.2 Bacteriocins and Hurdle Technology

After a lot of the experimental reviews, it was observed that the microorganisms cannot survive under different antimicrobial factors. So, keeping this problem in mind, the idea of hurdle technology came into existence in the food manufacturing units. Over 60 possible hurdles have been styled to expand food stability or quality (Leistner, 1999). This has established great importance in modern era. Bacteriocins can be used in combination with the hurdle technology to reduce the microbial load of food products.

4.2.1 Bacteriocins combined with various chemical compounds and antimicrobial drugs:

Bacteriocins like leucocin F10, Nisin, enterocin AS-48, when combined with Sodium Chloride (NaCl), enhanced the antimicrobial actions. The presence of NaCl also decreased the antilisterial activity. The effect of NaCl is because of the interference with ionic interactions between the highly charged functional groups and bacteriocin molecules which are involved in binding to the targeted cells (Choi et al., 2021). The reduction in the nitrite content of the food has been proven advantageous in the food industry, especially in case of meat-based products. When combined with nitrite, Nisin has decreased the botulinal formation (a toxin) in meat-based products. It also has an active effect against the clostridial endospores outgrowths. Input of nitrite has also enlarged the anti-listerial activity. Adding ethanol with Nisin can act simultaneously to decrease the growth of *L. monocytogenes* (Brewer et al., 2002). The development of *B. licheniformis* was inhibited by the combination of Nisin and monolaurin. Chelating agents can be used to stabilize and increase the self-life of the food products. They mostly penetrate the outer membrane of the bacteria by utilizing the Ca^{2+} & Mg^{2+} cations, which help stabilize the lipopolysaccharides, allowing the bacteriocins to reach the target (Saha, 2020).

4.2.2 Heat treatments along with bacteriocins

Bacteriocins and heat treatments can reduce the microbial load in food products. Heat and nisin act synergistically against *L. monocytogens* and *L. plantarum*. It also reduces the heat resistance capacity of *L. monocytogens* in milk-based products and cold-packed lobster meat (Krishnamoorthi et al., 2022)

4.2.3 Bacteriocins and Modified Atmosphere Packaging

The most advanced type packaging system i.e. Modified atmosphere packaging (MAP) is most commonly used in the food industry to increase the shelf life of fresh products. The increase in the shelf life of the food by MAP is based on the hindrance of the intrinsic food changes and prohibits the growth of the microorganisms. The dissolved CO_2 inhibits the growth of the microorganism. As was earlier reviewed, Gram-negative bacteria being sensitive to carbon dioxide, whereas lactic acid bacteria are resistant to carbon dioxide. However, they are not sensitive towards bacteriocins. So, combining bacteriocins with MAP gives more advantages in managing food spoilage (Yadav et al., 2020).

4.2.4 Bacteriocins and pulsed electric fields (PEF)

The microbial inactivation can also be achieved through pulse electric fields, a non-thermal process. This process has gained importance recently as an individual treatment or combination with bacteriocins. The treatment of PEF with bacteriocins for food preservation depends on factors like PEF treatments, microbial load on the product, bacteriocins, composition of the food products and various environmental factors (Heinz et al., 2002).

4.2.5 Non-thermal treatments along with bacteriocins

Several non-thermal treatments have been used for the preservation of food products. One of them is irradiation techniques. The broad spectrum of irradiation could be expanded when combined with bacteriocins. In this combined technique, the radiation dose will be lowered by lowering the unwanted effects on the food and increasing acceptance among the consumers. A study suggests that when pediocin is combined with low-dose irradiation, the antimicrobial effect on L. monocytogenes increases (Chen et al., 2004).

5. CONCLUSION & FUTURE SCOPE

Artificial preservatives are chemical substances that can cause severe health hazards. Natural preservatives offer great advantages as they are non-toxic and easily acceptable. One of them is bacteriocins. Bacteriocins are gaining importance in current research as they are adaptable and can be used as preservatives, growth promoters, alternative antibiotics, etc. It is regarded as one of the major solutions for food spoilage. Continuous use of antibiotics can lead to the disruption of the microbiome and can cause the transfer of the resistance gene. They have inhibitory activity against antibiotic-resistant bacteria. Many bacteriocins are available, and several studies indicate their usefulness in food preservation. Novel preservation techniques are available, giving new opportunities for the various applications of bacteriocins as part of the discussed hurdle technology. However, several unexplored areas include combination of bacteriocins with irradiation, ohmic heating, pulsed light, ultrasonication, etc. Until now, there are several commercially available bacteriocins, and luckily the, consumers demand less processed food with minimal use of chemical preservatives. In this case, bacteriocins can be considered a good option in the food industry. The main benefit of using bacteriocin is that it can preserve food products without hampering the sensory quality of the food.

There should be a search for new and modified bacteriocins which will have some unique properties and can be expanded for industrial applications. There should be a search for new bacteriocins-producing strains, the yield of the bacteriocin production should be increased, strains having new properties should be searched and should be used in place of synthetic preservatives, which are safe and have low cost production.

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