Bacteriocin: A Potential Biopreservative in Foods

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Bhuvaneswari Manivel and Subashini Rajkumar

Abstract

Biopreservation method focuses a great attention to food industry and consumers. Antimicrobial peptides were also termed as proteins or polypeptides produced by bacteria of both Gram negative and Gram positive, during their growth and possess antimicrobial activities. Even though bacteriocins are categorized as antibiotics, they are not. Bacteriocins are generally ribosomally synthesized; some are posttranslationally modified. They have a broader spectrum of activity to the closely related strains. Microbes produced bacteriocins during the primary phase of growth, but antibiotics are synthesized only as secondary metabolites. Bacteriocins are generally cationic and low molecular weight, are easily digested by intestinal enzymes and contain a surplus of arginyl and lysyl residue. They are amorphous in nature and showed helical structure when soaked in aqueous solution. Nowadays bacteriocins are widely used in food processing as natural preservatives, and the use of their metabolic products is generally recognized as safe (GRAS). The natural antimicrobial compound undergoes research in a genetic level as alternative to conventional antibiotics which will benefit both the consumer and the producers.

Keywords

Biopreservative · Bacteriocins · Foodborne pathogens · Food spoilage bacteria · Subtilin

B. Manivel (✉)
Department of Chemistry and Bioscience, Srinivasa Ramanujan Centre, SASTRA Deemed to be University, Kumbakonam, Tamilnadu, India

S. Rajkumar
Department of Microbiology, Shrimati Indira Gandhi College, Trichy, Tamilnadu, India

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6.1 Introduction

The antimicrobial technique in living framework are utilization of antimicrobial peptides by the intrinsic response of immune of numerous life structures vary from creepy crawlies to plants to people. The evolution of penicillin opens the entry for the utilization of remedial anti-infection agents by the therapeutic networks to follow up on different disease causing microorganisms. In the food processing industry, the utilization of antimicrobial substance to food processed had turned into a conventional weapon in the food conservation methods. Numerous Gram-negative and Gram-positive microscopic organisms synthesize various advanced substances of protein structure (it may be neither proteins nor polypeptides) having an antimicrobial action called bacteriocins. It has increased gigantic consideration as potential biopreservative. Bacteriocins have antitoxin properties; however bacteriocins are regularly not named as antimicrobials (Cleveland et al. 2001). Bacteriocin holds opposing views from most restorative antitoxins in being proteinaceous and having limited specificity against strain of the equivalent or strain of firmly linked species (Schillinger et al. 1996).

To keep up the nature of raw material, various physical and chemical properties of the products were maintained in food preservation to help provide stability and protection. The method of preservation aims to minimize or completely remove the disease cause in food. Numerous trends and safety concepts in food preservations indicate that various types of foodborne illnesses and food intoxication are on the rise today. Nowadays various commercial food products and easy uptake of food lead to high contamination. Currently broad vision and higher understanding capability in numerous microbial interactions raised the usage of biopreservative in the form of enzymes or a proteinaceous compound (Holzapfel et al. 1995).

To enhance the microbial safety in foods, various novel scientific trends were aimed on preservation of food using biological method. The application of microbial metabolic products suppresses or eliminates unwanted microorganism in food. The most prevalent bacteria are none other than lactic acid bacteria (LAB) found in various types of fermented food and generally named as GRAS (generally recognized as safe) microbes. Lactic acid bacteria promisingly remained as the most profitable culture classified as "food grade microorganisms" in different food systems. They are one of the probiotic cultures and offer different medical advantages, safe to man (Aymerich et al. 2000). Currently bacteriocin has extraordinary consideration as a novel food preservative because it is heat-stable and amenable to proteolytic inactivation.

6.2 Ecology of Bacteriocins

Early experimental studies showed that microorganisms have the ability to synthesize at least one or many bacteriocins considered as a specific characteristic feature. The multiplication and survival of a microbe in a specific environment eliminates the competitive pathogen of the same habitat. Few low molecular weight antibiotics or toxins are hydrogen peroxide, some enzymes which lyse bacteria, organic acids and diacetyl which have the same property as antimicrobial peptides even though it does not function like bacteriocin. The production of bacteriocin by bacteria plays an important role in bacterial population dynamics. On the basis of evolutional and environmental adaptations, various complex interactions take place in a mixed population.

Riley (1998) explained that examination of bacteriocins in natural environments, such as *Lactobacillus plantarum* in green olive fermentations, *Escherichia coli* in guinea pig conjunctivae, and *Streptococcus mutans* in the human oral cavity, has indicated that the competitive advantage is notably increased growth of bacteriocin-producing cells against bacteriocin-sensitive bacteria in the same environments (Riley and Wertz 2002).

The most widely recognized microscopic organism *E. coli* produces colicin. It contrasts from different bacteriocins of Gram-positive microbes in two diverse ways: firstly, development of pore in the cytoplasmic membrane and secondly host cell degradation and suppression of translation mechanism. Nearly 25 different types of colicins were identified from *E. coli*. Among this the majority of *E. coli* cells were highly opposing towards any one colicin, and the remaining population of *E. coli* cells were resistant to all types of colicin synthesized by cells of sensitive nature.

6.3 Bacteriocins

Bacteriocins are antimicrobial peptides which are produced ribosomally as polypeptides. They show strong bactericidal activity, while some are posttransitionally modified. They are produced by huge varieties of bacteria and some archaea and produce a wide variety of antimicrobial compounds. They are a heterogeneous group and use a specific antagonist against pathogenic bacteria. They are usually low molecular weight not often 10 kDa, and they are easily digested by proteases of mammalian gastrointestinal tract, indicating it is safe for human consumption. Commonly bacteriocins are cationic, amphipathic molecules that contain an excess lysyl and arginyl residues (Rodriguez et al. 2003). They are generally unstructured and easily incorporated into aqueous solutions. It forms a helical structure when exposed to solvents such as trifluoroethanol or combined with anionic phospholipid membranes. Likewise, they offer specific advantage in the survival area by eliminating the competitive pathogens in that habitat. Antimicrobials producing bacteria liberate various toxins either chromosomally synthesized or by means of plasmid acquiring the capability of eliminating predominant pathogens.

In recent years many bacteriocins are successfully identified by scientist. They usually inhibit the growth of organism of the same or closely related species. They also inhibit the growth of sensitive cells or kill them by interfering with the synthesis of cell wall or forming pores in the cell membrane (Settanni and Corsetti 2008). Many different microorganisms are known to produce bacteriocins. Bacteriocins are widely studied in gram-positive bacteria (antibiotics, pediocin-like bacteriocins) and gram-negative bacteria (colicin, microcins). Among them *Lactobacillus* and *Bacillus* species were the well-known producers (Lodewyckx et al. 2002).

Bacteriocins produced by *Bacillus* showed tremendous applications in inhibiting various pathogens when compared with other bacteriocins synthesized by other bacteria. Organisms such as bacteria, fungi or yeast may cause disease to humans as well as animals. When compared to conventional antibiotics, antimicrobial peptides of *Bacillus* showed tremendous advantage in food preservation and showed various environmental applications such as biocontrol of plant pathogens and plant growth promoters.

6.3.1 Antimicrobial Peptides Produced by Gram-Positive Bacteria

On the basis of various ecological as well as evolutionary aspects, the antimicrobial peptides synthesized by Gram-positive bacteria differ from Gram-negative bacteria. Likewise, antimicrobial peptides of Gram-positive bacteria are highly tremendous than Gram-negative (Jack et al. 1995). The inhibitory activities of lactococci towards other lactic acid bacteria are due to special molecule named as a proteinaceous substance or nisin (Heng et al. 2007).

There are numerous kinds of bacteriocin-producing organisms. Among those populations, *Bacillus* and *Lactobacillus* species highly engage themselves in food preservation. It can be discussed below.

6.3.2 Antimicrobial Peptides Produced by Lactic Acid Bacteria (LAB)

One of the major occurring bacteria is lactic acid bacteria. LAB is a Gram positive bacteria, non-aerobic, fastidious, non-respiring cocci or rods, non spore forming; obligate lactic acid fermenter, produce lactic acid as fermentative end product it showed catalase negative, devoid of cytochrome. In the 1900s based on their interaction in various foods, they are a group of bacteria considered to play a vital role as biopreservative. The ideal natural food preservative should have the following criteria: acceptably low toxicity, stable storage and processing time, efficient at low concentration and economically viable (Kindoli et al. 2012). Lactobacilli synthesize preservation factors commonly named as bacteriocin.

Among the numerous types of bacteriocin the discovery of nisin as a food preservative since ancient times. Bacteriocin has been widely used as commercial food preservative and their demand increased in the last century. Many bacteriocins have been isolated and characterized for their antibacterial activity against a wide range of food contaminating bacteria (Sakala et al. 2002).

6.3.3 Bacteriocin of *Bacillus*

Bacillus sp. is ubiquitous, commensal and transient organism in nature. It is one of the most well-known producers, and it is generally recognized as safe (GRAS) organism because it is degraded by proteases in the intestine of human being (Abdel-Mosheim et al. 2010). For this reason, bacteriocins from LAB and bacilli have a great potential as a natural preservative, replacing chemical preservative. The antimicrobial peptides synthesized by microbes possess selective benefits for their producer against competitive microorganism. The use of bacteriocin might be secluded from foods because the producer strain is often pathogenic in nature. Current development in recombinant engineering techniques involves the movement of bacteriocin production genes from the efficient donor of Gram-positive bacteria to Gram-negative bacteria of food grade microorganism.

Bacillus subtilis strains were one of the well-known producers as well as extensive synthesizer of diverse antimicrobial compounds. *B. subtilis* ATCC6633 produces several antibiotics, rhizocticin and lipopeptides, surfactin and mycosubtilin (Sutyak et al. 2008b). The bacteriocin-like substances of *B. subtilis* and LFB 112 exhibit maximum activity against both Gram-positive and Gram-negative bacteria causing a variety of diseases to animals. Bacteriocin-like substances (BLIS) can prevent the growth of many foodborne and spoilage bacteria such as *Staphylococcus aureus*, *E. coli, Salmonella pullorum, Pseudomonas aeruginosa, Clostridium perfringens.*

Bacillus subtilis GB 0365 is known to suppress various fungi such as *Botrytis, Fusarium* sp. and *Pythium* sp. In 1989, *Bacillus licheniformis* synthesize the antimicrobial peptide in an extreme environment while highly suppressing the activity of Gram-positive bacteria. The highest rate of antibacterial activity was observed in bacillocin synthesized by *Bacillus licheniformis* and also against some closely related species such as *B. cereus*. The probiotic *Bacillus clausii* O/C strain secretes proteinase perceptive antimicrobial substance and exhibits strong inhibition against *Staphylococcus, Enterococcus* and *Clostridium* sp.

6.3.4 Mode of Action and Structure of Subtilin

Bacillus subtilis produced a lantibiotic known as subtilin. It is categorized as a type A lantibiotic (Guder et al. 2000), cationic in nature and pentacyclic (Fig. 6.1). Its molecular mass is 3319.56 Da as revealed by the matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry (MS) (Stein 2005). The nisin Z leader sequence shares 57% amino acid identity with the leader sequence of subtilin. Likewise the pro-region of nisin Z has 61% identity with the pro-region of subtilin. Subtilisin has a strong bactericidal activity because it forms pores in the cytoplasmic membrane and cell wall precursors such as lipid II and pyro phosphate peptidoglycan monomers as a docking module (Martirani et al. 2002).

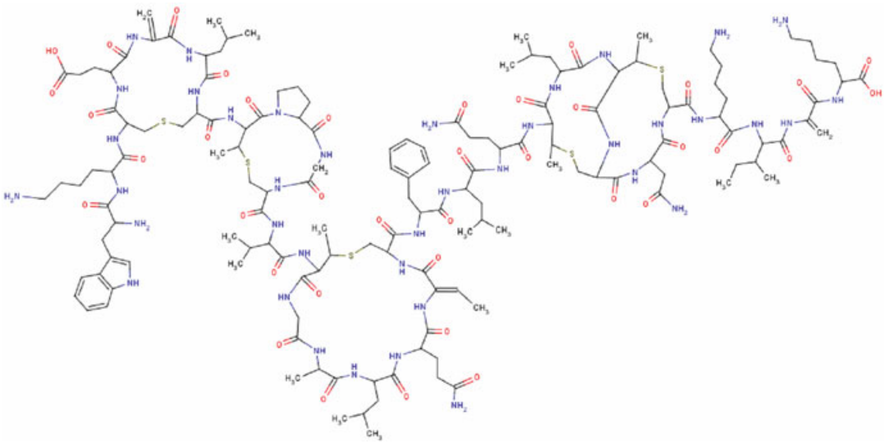

Fig. 6.1 Structure of subtilin

Subtilin molecular formula is –C148H227 N39 O38 S5. This structure is similar to nisin produced by *Lactococcus lactis* (Dodd et al. 1990), likewise, ericins from *B. subtilis* and *epidermin* from *Staphylococcus epidermidis.*

Subtilin is an antimicrobial peptide synthesized by *Bacillus*. The gene which encodes the subtilin harbours (Banerjee and Hansen 1988) nearly a 56-residue peptide precursor that is processed to yield the 32-residue mature peptide. The molecular weight of subtilin is 3319.56 Da as it was revealed by the matrix-assisted laser desorption/ionization time-of-flight MS (Stein 2005). The transformation occurs in precursors by a cyclic dehydration and cross-linking categories to obtain the complete antimicrobial peptide named as subtilin having a remarkable amino acid residues such alanine, methylanthionine, dehydroalanine and dehydrobutyrine. The precursor peptide possesses a leader region having an unusual hydropathic characteristic for an exported protein. Nearly 57% amino acid identity sequence of subtilin also shared by nisin sequence (Beasly and Saris 2004). Likewise, the initial regions of subtilin and nisin possess 61% identity. The structural similarity of subtilin is higher when compared with nisin, their specific operan which encode highly similar proteins.

Subtilin possesses bactericidal activity due to the presence of perforin in cytoplasmic membrane, and cell wall precursor like undecaprenyl pyrophosphate and lipid II act as a hydrophobic carrier module for peptidoglycan monomers as a docking module (Parisot et al. 2008).

6.3.5 Beneficial Role of *Bacillus* sp.

Most member of this *Bacillus* species shows heterogeneity in both phenotypic and genotypic characteristics. It has the ability to destroy a wide range of substrates obtained from both plant and animal sources (Lutz et al. 2006). These organisms are aerobic, Gram-positive, endospore-forming, rod-shaped bacteria that are characterized metabolically by catalase production. It has the ability to survive in the space for 6 years despite the harsh radiation, vacuum, temperature, etc. It is one of the most important probiotics that existed on the earth for a long time. It performs a vital role in suppressing a systemic pro-inflammatory and autoimmune disorder. It was the first bacterium responsible for determination of cell shape and synthesis of peptidoglycan. It was also identified for localization of peptidoglycan synthesizing enzymes, and it has the capability to synthesize a variety of key nutrients (vitamins, enzymes, carotenoids, lipids, etc.) at absorption site.

It is mainly used for the treatment of gastrointestinal and urinary tract disease. In such a way it functions as an immunostimulatory agent. *B. subtilis* produces carbohydrase (amylase) and protease enzymes. In 1960 this organism was certified by the USFDA (US Food and Drug Administration) due to its non-toxigenic and non-pathogenic nature. *Bacillus subtilis* is also precisely used in a variety of food products. *Bacillus subtilis* acts as a probiotic in everyone's healthy diet. It is highly incorporated in Korean food cheonggukjang. Most of the governmental and non-governmental organizations used dehydrated *Bacillus* culture as feed ingredients or as a silage additive for cattle feed. Spores of *Bacillus* are widely used as a probiotic food supplement.

It is used as a feed for human and feedstock for animals. *B. subtilis* was recognized as QPS (Qualified Presumption of Safety), and the European Food Safety Authority (EFSA 2007) declared it safe for livestock activities due to the lack of surfactant activities and lack of food poisoning toxins such as enterotoxin (Permtoonpatana et al. 2012). It acts as a potential source for the production of various enzymes such as proteases, amylases, some antibiotics, insecticides and also as rennet substitutes.

6.3.6 Categorization of Bacteriocins Produced by *Bacillus*

Like that of LAB bacteriocin, various categorization methods were now available for antimicrobial peptides of ribosomal synthesis. In 1993 Klaenhammer established the categorization of LAB bacteriocins. Similarly Van Belkum and Stiles (2000) and Nes et al.'s (2007) reclassifications were performed. Among different bacterial clades, the bacteriocin produced by *Bacillus* belongs to the lantibiotics, a category of posttranslational modified peptides. On the basis of peptide structure, genetic determinants and biosynthesis mechanisms, lantibiotics are considered as one of the most important antimicrobial peptides (Table 6.1).

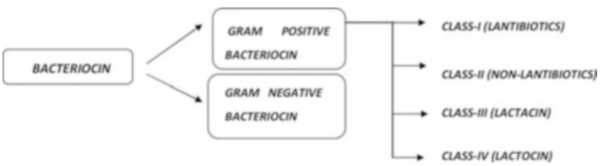

Table 6.1 Comparative features of conventional antibiotics with bacteriocins

Sl. no.	Conventional antibiotics	Bacteriocins
1	Complex ring structure	Proteinaceous in nature
2	Wide spectrum of activity	Narrow spectrum of activity
3	Widely used in clinical applications	More commonly used in medical applications
4	Absence of host cell immune response	Presence of host cell in response
5	Cell membrane is a target site	Cell wall is a target site
6	Specific target	Docking mode of interaction
7	Production cost is too cheap	Production cost is too high

Table 6.2 Classification of bacteriocins of *Bacillus* species and comparison with lactic acid bacteria (Adapted from Abriouel et al. 2011)

Proposed classification of bacteriocins of *Bacillus* species	Examples	LAB bacteriocins (Nes et al. 2007)
Class I. Posttranslationally modified peptides		Class I. Lantibiotics
Subclass I.I. Single-peptide elongated lantibiotics	Subtilin, ericin S, ericin A	
Subclass I.2. Other single-peptide lantibiotics	Sublancin 168, Mersacidin, Paenibacillin	
Subclass I.3. Two-peptide lantibiotics	Haloduracin, Lichenicidin	
Sub class I.4. Other posttranslationally modified peptides	Subtilosin A	
Class II. Non-modified peptides		Class II. Small linear peptides
Subclass II.1. Pediocin-like peptides	Coagulin, SRCAM 37, SRCAM 602, SRCAM 1580	Class II a
Subclass II.2. Thuricin-like peptides	Thurincin H, Thuricin S, thuricin 17, bacthuricin F4, cerein MRX1	
Subclass II.3. Other linear peptides	Cerein 7A, Cerein 7B, lichenin, thuricin 439	
Class III. Large proteins	Megacin A-216 Megacin A-19213	Class III. Large heat-labile bacteriocins

In 2011 Abriouel et al. proposed the classification of *Bacillus* bacteriocins (Table 6.2). Antimicrobial peptides that undergo different kinds of posttranslational modification belong to class I. It can be subdivided into four subclasses. Likewise, small (0.77–10 kDa), ribosomal synthesized, non-modified and linear peptides which are heat and pH stable belong to class II. This class II is subcategorized into three subclasses. The large proteins (430 kDa) with phospholipase activity such as megacins A-216 and A-19213 (Table 6.1) belong to class III.

6.4 *Bacillus* as Biopreservative

The bacteriocin synthesized from the *Bacillus species* suppresses various infections and are caused by most common foodborne pathogens such as *E. coli* and *Salmonella* sp. *Listeria monocytogenes* and *S. aureus* than bacteriocin produced from *Bacillus mycoides* (Sharma and Gautam 2008). The most common Gram-positive bacteria such as *Micrococcus luteus* and *S. aureus* are highly inhibited by two important species *B. subtilis* and *B. pumilus.*

As one of the industrially important species, *Bacillus* species has wide applications in the food industry. The following features depict that the *Bacillus* bacteriocins should be a better alternative to LAB bacteriocin.

In various food fermentation industries, numerous enzymes from *Bacillus* were most commonly used as a starter culture in food fermentation (Ananou et al. 2007). In food processing technology, the bacteriocins produced by *Bacillus* show strong antibacterial activity against various pathogens belonging to either gram-positive or Gram-negative bacteria. It is also effective against fungi. The antimicrobial peptides of *Bacillus* showed wide metabolic diversities such as heat stability, withstand stable pH, use of different organic solvents in food processing. The genetic map of *Bacillus* sp. was well known like *E. coli.* Hence it is very safe for the food industry to synthesize bacteriocin.

6.5 Applications of *Bacillus* Bacteriocins

Antimicrobial peptides offer much advantageous applications in various sectors because of the potential role in food preservation as well as in the medical sector as a therapeutic agent. In recent research, antimicrobial proteins synthesized from *Bacillus* contribute numerous applications in food preservations. It invokes specific interest in research because bacteriocin from Bacilli decreases the limitations of bacteriocin from Lactic Acid Bacteria. It shows wide inhibitory action against various gram-negative bacteria. It even kills fungi. Numerous beneficiaries suggested wide innovations in the field of human health, agriculture, livestock, and food preservation (Abriouel et al. 2011) (Fig. 6.2).

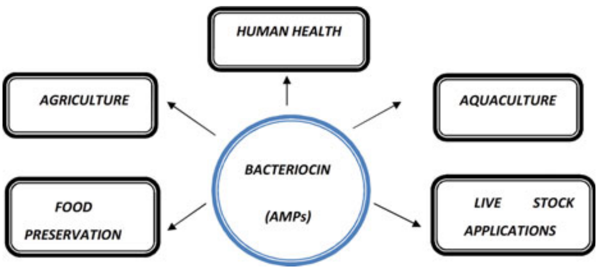

Fig. 6.2 Potential applications of bacteriocin in different sectors

6.5.1 Applications in Human Health

Bacteriocins are considered as a novel source for the control of microbial pathogens and also increase the bacterial resistance to conventional antibiotics (Lawton et al. 2007). Nisin is one of the nontoxic bacteriocins suggested as contraceptive agent. It has a potential to inhibit the growth and colonization of *Helicobacter* which causes peptic ulcer. Similarly, lantibiotic subtilosin shows highest spermicidal activity against spermatozoa from humans exhibiting antimicrobial activity against pathogens such as *Listeria monocytogenes, Gardnerella vaginalis and Streptococcus agalactiae* (Sutyak et al. 2008a). Bacteriocins of *Bacillus* were used to suppress the growth of other bacteria and mean while it offers advantage to microbes in fermenting ecosystem. It does not have the capability to cause vaginal irritation; hence it is more acceptable for human use. The subtilosin A bacteriocin produced by *Bacillus* has proven to be a strong antimicrobial agent against a wide range of pathogens including *Micrococcus luteus, Streptococcus agalactiae* and *Listeria monocytogenes*. It has a huge impact on vaginal pathogens while leaving the healthy microflora to remain intact. Likewise, *B. clausii* as a probiotic strain causes inhibition against *S. aureus, E. faecium* and *C. difficile* (Hill et al. 2009).

6.5.2 Applications in Livestock

Bacteriocin-producing bacilli predominantly act as probiotics in livestock to improve animal health and also inhibit pathogenic bacteria. In 2005 Stern et al. reported that bacteriocin of *Bacillus* sp. has tremendous applications in husbandry. *B. licheniformis* and *B. subtilis* in combination are used to prepare BioPlus 2B. The poultry intestinal pathogens such as *E. coli* and *Yersinia* were strongly suppressed by the spores of *Bacillus amyloliquefaciens*. It is widely used as a probiotic in various animal feeds (Ecobiol, Norel and Nature Nutrition). The non-toxigenic *Bacillus cereus* is used as an animal feed additive because of its probiotic activity. This strain has already been approved by the European Food Safety Authority (EFSA) for animal feed (EFSA 2007).

Antimicrobial proteins commonly used as growth promoters or therapeutic agents and also in animal research are a valuable tool. Nowadays conventional antibiotics were totally altered by bacteriocins in such a way that it reduces the antibiotic-associated problems such as presence of antibiotic residues in the environment and veterinary products and also induced the resistance frequency in bacterial species (Oppegard et al. 2007).

The antimicrobial proteins synthesized by Gram-positive bacteria commonly improved the livestock produced both in vivo and in vitro. Meanwhile, poultry farming, one of the major contaminants as *Salmonella*, was controlled by the use of antimicrobial peptides of *Bacillus*. Microcins produced by *E. coli* hold a promise in reducing the population of Salmonella species in broiler chickens. *Bacillus* species was also commonly used in poultry system as probiotic that showed a maximum decrease of pathogenic bacteria. Current report stated that when colicin synthesizing the bacteria was inoculated into rumen of cows, it highly reduces the amount of enteric pathogens in the animal. Likewise, lacticin produced *Lactococcus lactis* shows a maximum activity against streptococci and staphylococci in dairy cattle. Current report predicted that probiotic science capable of synthesizing bacteriocin increased the growth rate of swine similar to cattle.

6.5.3 Applications in Food

Currently bacteriocins are extensively used in the food industry especially on food products such as eggs, vegetable and meat. It is highly incorporated as starter cultures. The usage of *Bacillus* bacteriocins specifically focused on targeting food pathogens with special attention. Nowadays many foods are highly incorporated with chemical preservatives (Chen and Hoover 2003). Hence most of the consumers demand for natural foods or minimally processed food, stimulating great interest to antimicrobial agents such as bacteriocin. Dairy products such as milk and soft cheese are specifically preserved by using bacteriocins of *Bacillus*. The bacteriocin cerein 8A synthesized by *Bacillus cereus* 8A was used to control *Listeria monocytogenes* (Bizani et al. 2008). Bacteriocin-like substances synthesized by *Bacillus amyloliquefaciens* strain was used for the biopreservation of poultry milk (Halimi et al. 2010).

Bacteriocins are most resistant to physical factors, while during food processing, they can be neutralized by proteolytic enzymes. It shows higher inhibition to foodborne pathogens. Meanwhile *Bacillus* bacteriocins are considered superior to LAB bacteriocins (Zacharof and Lovitt 2012). It can also be used to enhance sensory properties and improve food quality. Moreover it is integrated into food as sodium acetate or sodium lactate. Apart from these, microbes were predominantly used in the production of various alkaline fermented foods. *B. subtilis* is the only strain which is highly incorporated into East Asian fermented food products (Hosoi and kiuchi 2003). Additionally, certain *Bacillus* species are widely used as inoculums for fermenting soybeans and condiment dawadawa (Terlabie et al. 2006). Likewise, subtilisin was incorporated into fermented soup condiment okpehe. Similarly, *B. subtilis* strain inhibited the growth of various *Bacillus* species (Bhuvaneswari et al. 2016).

6.5.4 Application in Aqua Culture

Marine animal associated microbes were the potent bacteriocin producers. Bacteriocins serve as an eminent tool to reduce the potential pathogens in sea food industry. Antimicrobial proteins totally exclude pathogenic bacteria in water bodies, but enhance the production of inhibitory compounds and also provoke the nutritional state of species by synthesizing the digestive enzymes. The antimicrobial proteins harboured by *Serratia, Pseudomonas, Stenotrophomonas, Photobacterium, Bacillus* and *Aeromonas. Vibrio* species from marine environment was highly screened for high molecular weight antimicrobial proteins like the compound called hamycin. Various bacteriocins like divercin, divergisin and piscicocin are commonly isolated from *Corynebacterium* (Suzuki et al. 2005).

6.5.5 Applications in Agriculture

Bacilli are commonly found in all sources especially in soil and plants. Majority of bacteriocins inhibit plant pathogens because they possess antibacterial or antifungal activity; hence they are used as biocontrol agents. For example, ericin S produced by *Bacillus subtilis* A1/3 is active against the causative agent of tomato bacterial canker *Clavibacter michiganensis*. From the rhizosphere of a healthy plant, *B. subtilis* 14B was isolated, and it produced Bac 14b, a BLIS active against *A. tumefaciens*. Hence, it is commonly used as a biocontrol agent for suppressing diseases in plants caused by *A. tumefaciens* (Hammami et al. 2009).

Most of the bacilli act as a plant growth promoter and also offer disease resistance in plants. From soya bean root nodules, *B. thuringiensis* strain NEB17 was isolated and used as a co-inoculant with *Bradyrhizobium japonicum* 532C; it enhances nodulations by producing polypeptide. Consequently, the antibacterial peptide thuricin was liberated by *Bacillus*. The introduction of thuricin in soybean caused structural deformities in root hair and also led to curling of root hair tip. The application of thuricin to any part of the plant stimulates the leguminous plant growth in a tremendous manner. The N-terminal amino acid sequence of thuricin was much correlated with other bacteriocins especially from the strain *Bacillus*. The antimicrobial peptides from this organism are thuricin S, thuricin H, bathuricin F4 and cerein MRX1. Meanwhile the other antimicrobial peptides possess the same activity like *Bacillus* and moreover induce the growth of plants.

6.6 Hurdle Technology in Biopreservative

Traditionally, multiple methods of food preservation has developed new ideas to hurdle technology, the presence of microbes in food is not an additive but it also provides some beneficial role. The hurdle technology focused much attention on low-fat content processed food for extending shelf life. The main concepts of hurdles during food safety are water activity, vacuum packaging, temperature, chemical preservative, water activity and UV. In addition to medical applications, bacteriocins are hostile to many important medical and veterinary pathogens. In particular, probiotic microbes liberate bacteriocin in human and animal intestinal tract; hence they protect gut microflora. They have the ability to kill a relatively narrow range of bacteria without causing any harm to natural microbiota, a unique feature when compared to other traditional antibiotics. However, they do not kill many pathogens, but they have the ability to play a very unique role. However, future studies should turn these bacteriocins into practical clinical substitutes to antibiotics and prove their predictable effectiveness, protection and affordability.

6.7 Conclusion

The bacteria from various sources have a capability to produce a variety of bacteriocins. They have a wide range of applications including both in the medical and food industry. To sustain the food quality in a safer manner various bacteriocin synthesizing cultures used as a starter or cocultures in various sectors. The high stability of *Bacillus subtilis* in extreme conditions makes this organism a suitable candidate for probiotic applications either in baked and pasteurized products or in other forms like tablets, capsules and powder. Common *Bacillus* bacteriocins such as subtilin, subtilisin and pediocin were widely applied as preservatives in various dairy products like cheese, kefir, kumis, etc. Further research is required to acquire knowledge in genetic mechanism for bacteriocin production and immunity. Moreover, pharmacological studies and the nature of the molecule after ingestion were required to establish the GRAS status. The use of bacteriocins in combination with natural technology could pave the way to replace the usage of chemical preservatives or could allow less severe processing treatments while still maintaining microbiological safety and quality in foods.

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