Bacteriocins and their technological applications: A review

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Abstract
The purpose of this study is to review the various aspects of Bacteriocins and their potential application in food and medicine sector with relevance to the challenges faced in the current era. Various studies done to explore the information on Bacteriocins along with their innate potentials in diverse sectors have been discussed in the review with appropriate examples. The employment of Bacteriocins as a natural food preservative is limited by various factors like effectiveness elimination of pathogen, expensiveness, etc. ([Chen and Hoover, 2006]). The past years have brought the Bacteriocins into lime light due to the emergence of considerable interest in their application as food preservatives.

Introduction
The need for scientific recognition is considered to be an essential factor since the significant contributions of Louis Pasteur and Robert Koch and this factor plays an important role in the controlling of microorganisms that are detrimental to the environment. The introduction of antibiotics with the recognition of penicillin discovered in 1929 by Alexander Fleming led to novel avenues that emphasized the therapeutic applications of antibiotics in the battle against disease-causing organisms. The increased exploitation of antibiotics has led to the development of resistance in the micro-organisms resulting in more potent drug resistant infections ([Vrancianu et al., 2020]). In such a scenario, one such alternative to the conventional antibiotics is the Bacteriocins.

The Bacteriocins are constituted by peptides or proteins that are ribosomally synthesized by the bacteria as a secondary metabolite in its fight for survival. These peptides or proteins are capable of either inhibiting or destroying other microorganisms ([Cotter et al., 2005; De Vuyst and Leroy, 2007]). They can possess narrow spectrum or broad spectrum activity and can inhibit taxonomically close bacteria or a wide range of bacteria based on their corresponding spectrum of activity ([Cotter et al., 2005; Mills et al., 2011]).

The past years have brought the Bacteriocins into lime light due to the emergence of considerable interest in their application as food preservatives. This ability of serving as a safe preservative is due to the fact that they can be easily digested by the human gastrointestinal tract ([Mills et al., 2011]). The employment of Bacteriocins as a natural food preservative neutralizes the demands for good quality as well as safe food without preservation using chemical substances. Despite the advantages, the application of Bacteriocins as a food additives is limited by various factors like effectiveness elimination of pathogen, expensiveness, etc. ([Chen and Hoover, 2006]).

The very first bacteriocin to be isolated was achieved in a study employing Gram-negative bacterium Escherichia coli and was named as colisin after isolation in 1925. Till date, colisins of E. coli remain to be one of the most extensively probed Bacteriocins. Several related studies on Bacteriocins have reported that these molecules constitute a diverse group of antibacterial peptides that are capable of posing significant cidal effects on the taxonomically close bacteria. The various modes of action involved are the inhibition of cell wall synthesis, permeabilization of the target cell membrane leading to its destabilization, or inhibition of...
RNase as well as DNase activity (Balciunas et al., 2013; Cleveland et al., 2001; Nes et al., 2007; Riley and Chavan, 2007; Riley and Wertz, 2002) [5, 9, 41, 45]. Several recent studies have reported that bacteriocin production is not completely restricted to the Gram-negative bacteria and were found to be produced by Gram-positive lactic acid bacteria (LAB). This group constituted by phylogenetically diverse microorganisms were found to be characterized on the basis of their morphological, metabolic as well as physiological properties and were reported to produce Bacteriocins with great application potential in industrial and medicinal sectors due to the GRAS status (generally recognized as safe bacteria) of the LAB (Cintas et al., 2001) [8]. In addition, the Bacteriocins from Gram-positive bacteria show a special inhibitory effect that is not directed against only bacteria within the same species as the bacteriocin producer but also against other species and/or genera different from the producer. Thus, the Bacteriocins produced by Gram-positive bacteria seem to possess a broader range of susceptible organisms and it makes these types of molecules more suitable for technological processes (Cintas et al., 2001; Gálvez et al., 2007; Nes et al., 2007) [8, 16, 41]. This review emphasizes on the various aspects of the Bacteriocins in relevance to the requirements of the current world.

Ecological perspectives of Bacteriocins

In the basis of evolutionary pattern, the innate ability to produce one or more Bacteriocins is considered to be a highly advantageous feature. In the study by Riley (1998) [46] that aimed in the examination of Bacteriocins in their natural environment, analysis was carried on Escherichia coli in the conjunctiva of guinea pig, Streptococcus mutans in the human oral cavity and Lactobacillus plantarum in green olive fermentations. The study reported that the competitive advantage was substantially increased in the case of bacteriocin-producing cells when compared to the bacteriocin-sensitive bacteria that existed in the same environment (Riley, 1998) [46]. In another study based on the case of the L. plantarum, bacteriocin-producing strain was employed for the fermentation of Spanish-style green olives and it was found that the bacteriocin producer remained at elevated levels over the course of the 12-wk fermentation. In the context of such studies, mathematical models have been devised for the evaluation of the interaction between the bacteriocin producing strain and bacteriocin sensitive strains. Several ecological models of Bacteriocins have been done with focus on colicins produced by Escherichia coli showing activity against other strains of E. coli and related members of the Enterobacteriaceae. The Colicins differ from Bacteriocins that are produced by the gram-positive bacteria and are capable of three general mechanisms of action that comprises of formation of channel in the cytoplasmic membrane, cellular DNA degradation and protein synthesis inhibition. It is has been estimated that around 30% of the natural population of E. coli are capable of producing Bacteriocins (Riley, 1998) [46]. About 25 types of colicin have been identified (Pugsley, 1984). It has also been brought to the limelight that about 70% of the cells an E. coli population are capable of exhibiting resistantance to any one type of colicin, and about 30% can demonstrate resistance against all colicins produced in a population (Smarda, 1992) [52].

Classification of bacteriocins

The Bacteriocins have been classified under various classes on the basis of various criteria like the type of producer, physical properties, molecular sizes, chemical structures and their mode of action mechanism. However, there is lack of a definite classification. In the initial years, the Bacteriocins were categorized into four classes by Klaenhammer in the year 1993 [31] (Klaenhammer, 1993) [31]. The class I comprises of lantibiotics that are characterized by very low molecular weight <5kDa and is constituted by lanthionine & its derivatives. The class II comprises of small thermostable peptides that lack lanthionine derivatives and weighs around 10 kDa in molecular weight. The class II is further divided into three subclasses such as IIa, IIb and IIc. The class III is constituted by compounds of high molecular weight >30 kDa with thermostable and the class IV is constituted by large peptides combined with carbohydrates or lipids (Balciunas et al., 2013; Klaenhammer, 1993) [5, 33]. As a contradiction to the classification by Klaenhammer, Cleveland et al. in 2001 [9] stated that the complex structures of class IV are resultant artifacts obtained due to partial purification and must not be assigned an individual class of Bacteriocins (Cleveland et al., 2001) [9]. Cotter et al. in 2005 [10] proposed a new classification comprised of two classes: the class I is constituted by lantibiotics and the class II consists of peptides devoid of lanthionine. The High molecular weight thermolabile peptides were separately categorized as bacteriolyins (Cotter et al., 2005) [10]. The current classification was proposed by Drider et al. in 2006 constituted by three main classes (Table I) that was segregated on the basis of their genetic and biochemical characteristics (Drider et al., 2006) [14].

<table>
<thead>
<tr>
<th>Bacteriocin</th>
<th>Producer</th>
<th>Reference</th>
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<tbody>
<tr>
<td>nisin</td>
<td>Lactococcus lactis</td>
<td>(Microbiol. and 1981, n.d.) [37]</td>
</tr>
<tr>
<td>epidermin</td>
<td>Staphylococcus epidermidis</td>
<td>(Allgaier et al., 1986) [11]</td>
</tr>
<tr>
<td>lactacin 481</td>
<td>L. lactis</td>
<td>(Piard et al., 1992) [42]</td>
</tr>
<tr>
<td>mersacidin</td>
<td>Bacillus subtilis</td>
<td>(Altena et al., 2000) [12]</td>
</tr>
<tr>
<td>actagardin</td>
<td>Actinoplanes sp.</td>
<td>(Sahl and Bierbaum, 1998) [49]</td>
</tr>
<tr>
<td>cinnamycin</td>
<td>Streptomyces cinnamoneus</td>
<td>(Sahl and Bierbaum, 1998) [48]</td>
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Class II

They are a Heterogeneous class of small thermostable peptides.
Enterococci are produced during the stationary phase of the cells (Ghammi et al., 2020) [18]. It has been analysed that under the situation of resource scarcity created by microorganisms, the producer strains secrete archaeocins that lyse the target cells and reduces the competition within the local environment (Riley and Chavan, 2007; Riley and Wertz, 2002) [45].

Bacteriocins of Gram-negative Bacteria

The colicin obtained from E. coli was identified by Gratia as an antimicrobial protein in 1925 and this was the first described one in the family of Bacteriocins and since then it has dominated several studies up to the current period. Several Gram-negative bacteria possess ability to produce colicin-like proteins. Marcescins of Serratia marcescens, alveicins of Hafnia alvei, Klebicins of Klebsiella pneumonia, Cloacins of Enterobacter cloacae and pyocins of Pseudomonads are important representatives of Bacteriocins produced by other Gram-negative bacteria. Most of the Bacteriocins in this group are relatively enormous and heat-labile peptides. (Balcíunas et al., 2013; Cleveland et al., 2001; Nes et al., 2007.; Riley and Chavan, 2007; Riley and Wertz, 2002) [5, 9, 41, 45, 47].

Bacteriocins of Gram-positive Bacteria

Gram-positive bacteria are also renowned producers of a wide variety of Bacteriocins. The non-toxic property on the eukaryotic cells as well as a broad spectrum of bacteriostatic ability makes the Bacteriocins produced by the Gram-positive organism, a unique useful tool potential for industrial as well as medicinal applications. The lactic acid bacteria (LAB) are a group of phylogenetically diverse Gram-positive bacteria that have been characterized by certain common morphological, metabolic as well as physiological properties. These organisms have attracted much interest owing to their GRAS (generally regarded as safe) that is a necessary element for marking the administrable for human consumption (Balcíunas et al., 2013; Cintas et al., 2001; Cleveland et al., 2001; Gálvez et al., 2007, 2008; Nes et al., 2007.; Riley and Chavan, 2007) [5, 8, 9, 16, 17, 41, 45].

Mode of Action

The Bacteriocins possess distinct mechanisms and can be classified as those with bactericidal effect with or without cell lysis and bacteriostatic effect with ability to inhibit cell growth (da Silva Sabo et al., 2014) [12]. A majority of the Bacteriocins that are produced by LAB inhibit the Gram-positive bacteria by targeting the cell envelope-associated mechanisms (Cotter et al., 2013) [11]. Several lantibiotics and several Bacteriocins of class II target the Lipid II which occurs as an intermediate in the peptidoglycan biosynthesis machinery that occurs within the bacterial cell envelope and in this inhibition of the peptidoglycan synthesis occurs (Breukink and de Kruijff, 2006) [16]. The Lipid II is employed as a docking molecule by other Bacteriocins for facilitating the formation of pore which results in variation of the cytoplasm membrane potential leading untimely death of the death (Machaidze and Seelig, 2003) [34]. Certain types of Bacteriocins capable of destroying the cells by the binding to the cell envelope-associated mannose phosphotransferase system (ManPTS) which results in the formation of formation of pores in cell membrane (Cotter et al., 2013) [11]. Certain Bacteriocins are capable of killing their target cells by the inhibition of gene expression (Parks et al., 2007; Vincent and Morero, 2009) [43, 56] and production of protein (Metlitskaya et al., 2006) [38].

Microbial Origin of Bacteriocins

The Bacteriocins are fundamentally divided in three groups based on the source from which they are derived:

Bacteriocins of Archaea

The Archaea are a group of bacteria capable of synthesizing their own distinct family of bacteriocin-like antimicrobial peptides and these compounds are named as archaeocins. The halocin S8 derived from halobacteria is a short hydrophobic peptide composed of 36 amino acids and was the first discovered member of the archaeocin family. These proteins are produced during the stationary phase of the cells (Ghammi Table 2: Important bacteriocins produced by Lactic Acid Bacteria.

<table>
<thead>
<tr>
<th>Bacteriocin</th>
<th>Producer</th>
<th>Reference</th>
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<tbody>
<tr>
<td>Nisin A</td>
<td>Lactococcus lactis</td>
<td>(De Vuyst and Leroy, 2007) [13]</td>
</tr>
<tr>
<td>Lactococcin DR</td>
<td>Lactococcus lactis ADRIA 85L030</td>
<td>(Dufour et al., 1991) [12]</td>
</tr>
<tr>
<td>Estreptococcin A-FF22</td>
<td>Streptococcus pyogenes FF22</td>
<td>(Hynes et al., 1993) [38]</td>
</tr>
<tr>
<td>Lactocin S</td>
<td>Lactobacillus sakei L45</td>
<td>(Nissen-Meyer et al., 1992) [42]</td>
</tr>
<tr>
<td>Pediocin PA1</td>
<td>Pediococcus acidilactici PAC-1.0</td>
<td>(Henderson et al., n.d.) [23]</td>
</tr>
<tr>
<td>Leuoccin A-UAL187</td>
<td>Leuconostoc gelidum UAL187</td>
<td>(Hastings et al., 1991) [21]</td>
</tr>
<tr>
<td>Mesentericin Y105</td>
<td>Leuconostoc mesenteroides Y105</td>
<td>(Hechard et al., 1992) [22]</td>
</tr>
<tr>
<td>Acidocin A</td>
<td>Lactobacillus acidophilus TK9201</td>
<td>(Kanatani et al., 1995) [10]</td>
</tr>
<tr>
<td>Bavarian A</td>
<td>Lactobacillus bavariicus MI401</td>
<td>(Larsen et al., 1993) [35]</td>
</tr>
<tr>
<td>Curtacina A</td>
<td>Lactobacillus curvatus LTH1174</td>
<td>(Tichaczek et al., n.d.) [94]</td>
</tr>
<tr>
<td>Plantaricin S (Plsα and Plsβ)</td>
<td>Lactobacillus plantarum LCPO10</td>
<td>(Jiménez-Díaz et al., 1995) [23]</td>
</tr>
<tr>
<td>Termophilin 13 (ThmA/ThmB)</td>
<td>Streptococcus thermophilus SPl13</td>
<td>(Marciset et al., 1997) [19]</td>
</tr>
<tr>
<td>Piscicolin 61</td>
<td>Carnobacterium piscicola LV61</td>
<td>(Holck et al., 1994) [25]</td>
</tr>
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~ 70 ~
The LAB are characterized by their ability to produce lactic acid by their fermentation pathway thereby by conferring them the name “lactic acid bacteria”. In the process of fermentation, a member of the LAB converts about 50% of carbon from the sugars into two isomers of lactic acid. Due to their safe nature and valuable metabolic products such as organic acids, diacetyl, acetoin, reuterin, reutericyclin, hydrogen peroxide, antifungal peptides, and Bacteriocins, the LAB have a great importance in medicinal and food applications (Güllüce et al., 2013) [19].

Biological features of bacteriocins

All the Bacteriocins are proteinaceous in nature and are synthesized by the ribosomes of the producer microorganisms in accordance to the genetic code that might have been occasionally be placed on plasmids, the parental chromosome or the mobile elements called transposons (Cintas et al., 2001; Cleveland et al., 2001; Riley and Wertz, 2002) [8, 9, 47]. The Genetic organization of the bacteriocin gene clusters are constituted by a functional operon comprising of the structural gene, the gene encoding immunity protein, the genes responsible for processing as well as transport of the bacteriocin, and, in certain cases, the genes coding posttranscriptional modification enzymes. Moreover, the bacteriocin operons also harbour the gene encoding the gene encoding for response regulator (RR), preinduction factor (IF) and the histidine protein kinase (HPK) gene (Cleveland et al., 2001) [9]. The activation of the structural gene marks the initiation of the bacteriocin production and this result in the production of biologically inactive precursors or prepeptides commonly referred to as the preproBacteriocins. Then, other related segments of the bacteriocin operon sequentially become operational and the process consequently results in the releasing of the mature Bacteriocins possessing antimicrobial activity (Cintas et al., 2001) [9].

Recent studies have also pointed out that the induction factors (IF or pheromone) which are a bacteriocin-like peptide composed of 19-26 amino acid residues length with low molecular weight and cationic nature possess great importance in the regulation mechanism (Cintas et al., 2001; Cleveland et al., 2001; Gálvez et al., 2007; Riley and Wertz, 2002) [8, 9, 16, 47]. The immunity of the bacteriocin producer strain to their own product brings out a clear demarcation between the Bacteriocins and antibiotics. In the concept of this phenomenon, a producer strain is capable of protecting itself from the toxicity of its own Bacteriocins. This protective mechanism is dependent on a variety of bacteriocin-specific immune based proteins. These kinds of proteins are encoded by gene sequences of close genetic proximity to the bacteriocin structural/processing genes and are commonly localized on the same operon. The characteristic feature of the immunity proteins are that they have high isoelectric point values in the range of 7-10, small sizes with 51-154 amino acid residues, and putative transmembrane α-helices. This type of characteristic feature facilitates the binding of these immunity conferring proteins to the cytoplasmic membrane of the host cell (Cintas et al., 2001; Cleveland et al., 2001) [8-9]. Resistance to Bacteriocins occur in the strains due to spontaneous or induced mutations leading to alterations in the membrane and cell wall. Modifications occur in the bacteriocin receptors, fluidity, membrane lipid composition, electrical potential and load or cell wall thickness as a result of the mutations (Cintas et al., 2001; Cleveland et al., 2001; Nes et al., 2007) [8, 9, 41]. Despite the lack of knowledge on the exact mechanism for resistance to bacteriocin, Van Schaik et al. has stated that the mutational changes on the cell surface of the resistant strain can possibly occur due to exposure of cell to low concentrations of Bacteriocins as a part of developing adaptive response to certain internal or external stress factors (Schaik et al., n.d.) [40].

Technological applications

The usage of the Bacteriocins in diverse technological applications fundamentally depends on their antimicrobial effects as well as on the value of antimicrobial activity required for the development of novel sophisticated strategies. The rapid emergence and spread of infections caused by multi-resistant bacteria has increased the consideration for research studies aimed in obtaining alternative strategy capable of battling against the infections. Bacteriocins exhibiting broad spectrum antimicrobial activity are a promising natural antimicrobial agent for several industrial applications (Balciunas et al., 2013; Cintas et al., 2001; Cleveland et al., 2001; Gálvez et al., 2007, 2008; Mills et al., 2011; Riley and Chavan, 2007; Riley and Wertz, 2002) [5, 8, 9, 16, 17, 38, 45].

Bacteriocins in Medicine

The field of Modern medicine has been threatened with the drastic increase in the emergence of antibiotic-resistant pathogens. The undesirable effects of the new-generation antibiotics are due to its high toxicity and therefore the need for alternative strategies is of ultimate importance. Bacteriocins possesses great calibre for serving as candidates for antimicrobial agents in human health and their applications have more advantages in comparison to the traditional antibiotics. The benefits of Bacteriocins are due to low-toxicity, presence of various types in nature, high target-specific mechanism and effectiveness even at nanomolar concentrations (Balciunas et al., 2013; Riley and Wertz, 2002) [5, 47].

Bacteriocins in Food sector

The Bacteriocins produced by LAB are currently the only Bacteriocins that are employed in preservation of food and in the production of fermented foods. The LAB has been employed for the fermentation of foods since centuries and are marked with the GRAS (generally regarded as safe) status according to the U.S. Food and Drug Administration (FDA). The important bacteriocins by LAB are given in Table II. This status permits their usage in the fermentation of foods without the requirement for an additional regulatory approval (Kanatani et al., 1995) [30]. Nisin is considered to be the first bacteriocin that was isolated as well as approved for application in the food industry, specifically for the prevention of Clostridium botulinum spores in the cheese spreads all over England. Various Attempts have been made for the utilization of Nisin A as an alternative to Nitrite, but these have not been able to produce successful results. Various Bacteriocins like the Carnobacterium, Pediococcus, Leuconostoc and Lacobacillus sp. have been reported to possess great potential for application in meat products (Stiles et al., 1991) [53]. Pediocin is a pure bacteriocin that is derived from Pediococcus and this bacteriocin has been utilized for the inhibition of growth of pathogenic bacteria in fully cooked meats subjected to storage refrigeration (Ladha et al., 2020) [32]. In a study, Crude forms of bavarcin A extracted from the
Lactobacillus bauaricus was compared with benzoate support solution and controls with no added preservatives and it was found to extend the shelf life of brined shrimp by about 16 days (Hjo’ et al., 1995) [24].

Bacteriocins in packaging film
The application of Bacteriocins in the industries by incorporating them into packaging films has been an area focussed in research for the last decade for the purpose of controlling food spoilage and in fighting against pathogenic organisms. This type of Antimicrobial agent incorporated packaging aids in the prevention of microbial growth on the surface of the food due to direct contact of the package with the surface of foods as in the case of packaged meat and cheese. (Appendini et al., 2002) [3]

Bacteriocins against Phytopathogens
The Bacteriocins named carotovorcin and serracin P obtained from Erwinia carotovora subsp. carotovorae and Serratia plymuthica respectively were analysed to structurally resemble the phage tails and were said to be induced by the effect of agents capable of damaging DNA (Muriana’t And and Klaenhammer, 1991; Science and 1992) [40, 50]. Glycinicin A produced by Xanthomonas campestris is considered to be the best described bacteriocin so far (Nissen-Meyer et al., 1992) [42]. This bacteriocin is a heterodimer composed of two polypeptides and was found to exhibit potential activity against phytopathogenic bacterial strains of Xanthomonas (sciences and 1984, n.d.) [51].

Future perspectives
A considerable interest has risen in the development of diverse combinations of antimicrobial agents available for developing a consortium capable of effective responses. This type of an approach basically involves combining of multiple available antimicrobial factors and is referred to as “hurdle technology”. The hurdle technology based on the application of bacteriocin involves the combination of one bacteriocin with another or other types of naturally derived antimicrobials, chemicals agents or admission to particular treatments (Gálvez et al., 2007) [10]. The commonly employed components are the combination of Bacteriocins with chemical substances like sodium chloride, organic acids and their salts, chelating agents, natural antimicrobials like essential oils and phenolic compounds comprising of thymol, terpineol, caffeic acid, carvacrol, eugenol, p-coumaric acid, Bacteriocins, non-bacteriocin antimicrobial proteins as well as peptides and physical treatments comprising of pulsed electric fields, heat treatments, high hydrostatic pressure, modified atmosphere packaging and other non-thermal treatments. Moreover, several efforts have provided promising results for the development of novel hurdles with a high efficiency rate for application in the near future (Gálvez et al., 2007) [10]. On the other hand, employment of computational methods for research on bacteriocin has drastically risen currently. The BAGEL and BACTIBASE are considered to be the most well-known examples for web databases and these facilitate access to computational tools for bacteriocin genome mining, similarity search engines like BLAST, FASTA, SSEARCH; sequence alignment tools like MUSCLE, CLUSTALW, T-COFFEE; physicochemical profile analysis, hidden markov models as well as for structure prediction tools (Gálvez et al., 2007; Hammami et al., 2010; Jong et al., n.d.; Mills et al., 2011) [16, 20, 29, 38].

Conclusions
The Bacteriocins serve as excellent candidates for various applications in both the food industry as well as in the medical sector. They are considered to be a diverse group of antimicrobial proteins/peptides produced by different types of bacteria as a secondary metabolite capable of targeting different bacteria to reduce competition by other microorganisms in a nutrient limited environment. With the increase in the case of drug resistant pathogen, the Bacteriocins can serve as a potential agent alternative to the conventionally employed antibiotics. Hence the Bacteriocins possess extreme potential in the field of medicine. The Bacteriocins also possess potential in the field of food processing and preservation of foods. The demand for natural and microbiological methods as a satisfactory approach in the food sector is capable of solving economic losses occurring due to microbial spoilage of raw materials and food products, reducing the incidence of food borne illnesses, and also in satisfying the food requirements of the growing world population. However further researches are required to explore the diverse capacities of the bacteriocin which can be suggestive of their application in several sectors.

References

~ 72 ~
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