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Bacteriocins: Limiting Factors to Optimum Activity

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Abstract Bacteriocins are described as ribosomally synthesized antimicrobial peptides lethal to bacteria other than the producing strain. They are the most abundant of antimicrobial compounds produced by bacteria. These antimicrobial peptides offer an advantage over by targeting specific organisms and are generally regarded as safe for humans. The crude bacteriocins have been found to be affected by the presence of proteolytic enzymes like trypsin, temperature, pH, salts, and ions like copper or iron. These antimicrobial agents are gaining attention not only as alternative therapeutics in the pharmaceutical industry but also as a bio-preservative in food industries and in agriculture for control of bovine mastitis pathogens. These applications fundamentally depend on their antimicrobial effects and a vast understanding of their activity and factors inhibiting their mode of action. In this review factors perceived to be consequential to either activating, inactivating or maintaining the optimal activity of bacteriocins were identified and discussed. This comprehensive review delved into these factors with the aim of in-depth understanding of bacteriocins and their application for extensive exploitation. The remits of this detailed review include aspects of re-assuring public faith in bacteriocins and providing adequate information to users on their activity under the various condition in order to make informed choices before use. This review will help in restoring confidence in bacteriocins as a substitute to conventional antibiotics presents a considerable commercial challenge to the pharmaceutical industry. This indulgence will help develop innovative strategies towards the industrial application of bacteriocins.

Keywords: *bacteriocins, antimicrobial peptides, antimicrobial activity, bacteriocidal spectrum*

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1. Introduction

All domains of life including Bacteria, Archaea, and Eukaryote have antimicrobial peptides (AMPs) [58]. An antimicrobial peptide or protein that is produced by bacteria and kills other bacterial strains is referred to as a bacteriocin [75]. Additionally, in a review by Sang & Blecha, [58], antimicrobial peptides are ubiquitous, gene-encoded natural antibiotics. These peptides have gained recent attention in the search for novel antimicrobials effective against the infectious disease.

Antimicrobial peptides including defensins and cathelicidins, provide a coordinated protective response against infection in multicellular organisms and principally, they are a component of innate immunity of invertebrates. Contrary, in unicellular organisms, antimicrobial peptides, such as bacteriocins, function to suppress and kill competitor species of bacteria by disruption of membrane integrity. Thus they are thought to be less likely to induce resistance. With this ability, AMPs are novel antimicrobial drugs [58].

Bacteriocins possess antibiotic properties [11]. However, they bacteriocins are not usually termed antibiotics in order to avoid misperception and concern with therapeutic antibiotics [13]. Originally, bacteriocins were called 'colicins' and were defined as bacteriocidal proteins characterized by lethal biosynthesis with a very narrow range of antimicrobial activity and adsorption at specific cell envelope receptors [19,43,51]. Cascales et al. [8], described colicins as proteins produced by *Escherichia coli* and toxic for some of its strains. Colicins are produced by *E. coli* strains carrying a carcinogenic plasmid. This plasmid bears the genetic determinants for its synthesis, immunity, and release. Further, Cascales et al. [8] reported that the lethal action of colicins is exerted by first binding to specific receptors on the outer membrane proteins used for the entry of specific nutrients.

According to Cleveland et al. [11] Bacteriocins are ribosomally synthesized antimicrobial peptides produced by one bacterium that is active against other bacteria, either in the same species (narrow spectrum) or across genera (broad spectrum). The description bacteriocin was first brought to the light by André Gratia in 1925. Gratia [25] initial discovery described the antagonism action exerted by *Escherichia coli* V towards *E. coli* Ø. Later, Cascales et al. [8] showed that the antagonistic effect was caused by a bacteriocin known as colicin V or microcin V. the term bacteriocin was proposed in 1953. It described the definition of colicin type bacteriocins since they were

the most studied then. This definition of bacteriocin had a narrow bacteriocidal spectrum with lethal biosynthesis, intra-specific activity and attachment to cell receptors as depicted by Tagg et al. [65].

Bacteriocins are commonly observed as having the ability to act on bacteria closely related to the producer hence a narrow bacteriocidal spectrum [29,65]. This generalization, however, is in need of modification since nisin, for example, has a larger spectrum of antimicrobial effect and effective against strains such as Listeria and Clostridium, [16]. In general, the bacteriocidal spectrum of bacteriocins remains relatively narrow because the bacteriocins produced by Gram positive bacteria cannot kill Gram negative strains under normal growth conditions [29]. Tagg et al. [65] reported that bacteriocins characteristically exert their antimicrobial action on species closely related to the producer and that action subsequently kill the competitors so that the producer may thrive.

Bacteriocins are reported to be a diverse group of proteins and peptides [36,58]. For example, lantibiotics belong to a bacteriocin group that is post-translationally modified while contains the non-proteinogenic amino acid lanthionine [42].

Ribosomal synthesis is responsible for the production and secretion of these proteinaceous antibacterial compounds by all main lineages of eubacteria and even in archaebacteria [42]. Halophiles being a key example as reported by Riley and Wertz, [52] and Torreblanca et al. [71]. A single species of bacteria may produce numerous kinds of which are ribosomally synthesized in the host. The producer strain possesses specific self-protection machinery against its bacteriocin [43]. These compounds show variability in biochemical properties, molecular weight, activity spectra and mode of action confirming that they are heterogeneous [36].

The inhibitory effects of bacterially produced compounds were made in 1933. These compounds were present in milk and though not known at the time, the inhibitory effects were due to bacteriocin [74]. Today, this bacteriocin is known as nisin. It is the most widely used and approved food preservative in more than 50 countries in England [12,16].

Initially, bacteriocin research majorly focused on Gram negative bacteria with little focus directed to Gram positive bacteria such as lactic acid bacteria [12,54]. In Cotter et al. [12], we find that the introduction of Nisin into the market in 1953 and its utilization in food applications resulted in a shift of bacteriocin research from Gram negative towards Gram positive bacteria. This drastic shift in research became popular making apparent problems with the original definition of bacteriocins. That is, the biosynthesis of bacteriocins was not lethal in Gram positive bacteria and neither did it show intracellular activity. To add, a wider bactericidal spectrum was observed in some bacteriocins [65]. To date, no bacteriocin can match the scale of the introduction of nisin in the international market. In Klaenhammer, [35] and Riley and Wertz, [52], it was predicted that 99% of all bacteria produce at least a bacteriocin. Currently, discoveries and characterization of new bacteriocins are continually being observed in various bacteria.

In microbial ecology, the production of antimicrobial substance is an important factor. Most of these substances play a key role in bacterial interactions, among them bacteriocins are highly specific and efficient antagonist as reported by Sahl, [57]. The most abundant of all the antimicrobial compounds produced by bacteria are bacteriocin. According to Reddy et al. [50], antimicrobial peptides help to protect humans against bacteria, fungi, yeast, viruses, and cancer cells because they form part of the innate immune system. In plants too, they offer natural defense mechanism and safeguard them against numerous pathogens. In addition, antimicrobial peptides serve as weapons in a microbiological war over inadequate resources in the micro-ecological environment [50].

These antimicrobial agents are gaining more and more attention not only as an alternative therapeutics for the prevention and treatment of infections in the pharmaceutical industry but also in food industries as a biopreservative as well to avoid deterioration and spoilage of food [3,24].

In this respect, the quick incidence and spread of resistant bacterial pathogens explicitly show the dire need for intensified research purposing to find alternative approaches to combating these infections. Focusing on bacteriocins, this review explored factors limiting optimal antibacterial activity these peptides produce by microbes with the aim of in-depth understanding of bacteriocins for extensive exploitation.

2. Literature Review

The potential use of bacteriocins for various technological application is currently on the rise [17]. Cleveland et al. [11] reported that bacteriocins produced by LAB used in food preservation become inactivated by digestive proteases, have a miniature effect on the gut microbiota and are usually pH and heat tolerant. Further, they are recognized as generally safe, have a broad antimicrobial spectrum against foodborne pathogenic and spoilage bacteria and show a bacteriocidal mode of action.

Studies on bacteriocins indicate that application of bacteriocin with these features in food industry extend the shelf life of foods, reduce food-borne pathogens transmission via the food chain, provide superfluous protection during temperature abuse conditions, moderate the application of chemical preservatives and allow the application of heat treatments that are less severe without compromising with the food safety [11,55]. These features further aid in healthier preservation of vitamins and food nutrients and retention of organoleptic properties of foods [23].

In food processing applications, bacteriocins are either added as ex-situ produced preparations or inoculation with bacteriocinogenic strains as reported by [23]. Bacteriocins, the antimicrobial agents in the food matrix can then show their specific activity. In many cases, the processing steps and the natural microbiota complex and non-stable nature. Thus, the bacteriocins have to pass all the limiting factors to exert their activity [27].

Bacteriocins that show broad-scale antimicrobial activity are thought of as promising natural antimicrobials for many industrial applications in this manner. Human health and food industries have recently dominated the

related studies and many prosperous improvements have been done to date [26].

Bacteriocin as one of the biological products of micro-organisms possesses specific characteristics that distinguish them from other types of molecules synthesized biologically [6]. These characteristics help to establish biological properties such as synthesis, mode of action, resistance mechanisms and immunity of bacteriocins from various sources. In the producer microorganisms, all bacteriocins are synthesized by the ribosomes due to their proteinaceous nature [1].

Abriouel et al. [1] in their review of diversity and applications of *Bacillus* bacteriocins reported that bacteria that belong to the genus *Bacillus* produce a wide variety of arsenal of antimicrobial substances such as peptide and lipopeptide antibiotics, and bacteriocins. Most of the *Bacillus* bacteriocins belong to the lantibiotics, a group of post-translationally modified peptides. These *Bacillus* bacteriocins are increasingly becoming more important due to their sometimes broader spectra of inhibition compared to most of the LAB bacteriocins which include Gram-negative bacteria, yeasts or fungi. Kiuchi & Hosoi, [34] reported that in Natto, an East Asian fermented food, *Bacillus subtilis* strains are used. Terlabie et al. [67] also reported that *B. subtilis* strains are used as a starter culture for fermenting soybeans or mesquite seeds.

In food processing such as diary, beverage and meat products Lactic acid bacteria (LAB) are extensively used. Their major role is to inhibit the growth of spoilage and pathogenic bacteria present in food [5]. These LAB are involved in the production of a variety of antibacterial agents such as organic acids, diacetyl, hydrogen peroxide and bacteriocins [73].

LAB are characterized by being cationic, hydrophobic or amphiphilic molecules [63]. They are composed of 20 to 60 amino acids residues. The primary receptor for bacteriocins of LAB are anionic lipids of the cytoplasmic membrane for initiation of pore formation. On the basis of their molecular mass, thermo stability, enzymatic, sensitivity, and presence of post-translational modified amino acids and mode of action, bacteriocins can be classified into 4 groups [63].

Bacteriocin production and immunity are encoded for by genes are usually organized in operon clusters. These gene clusters for bacteriocins are located on the chromosome, plasmid, and transposons as in cases of subtilin, diverging and nisin respectively [49]. Bacteriocin molecules auto-regulate their own biosynthesis via signal transduction by apparently acting as an external signal [18].

Ogunbanwo et al. [45] reported that the production of bacteriocins may prove advantageous for producer organism in a mixed fermentation environment to dominate the microbial population. The production of bacteriocins occurs not only during late logarithmic or early stationary phase but throughout the experimented growth phase [30]. Maximum activity of Bacteriocin has been observed on organism belonging to the same species. Jack et al. [29] reported that *Lactobacillus* bacteriocins, for example, shows less activity towards gram negative bacteria species. Some of the resistant gram negative cells of *E. coli*. *Erwinia carotovora*, *Pseudomonas aeruginosa* and *Serratia marcescens*. However, transforming the gram negative cells to spheroplast or by the use of chelating agents such as EDTA can make these strains sensitive to the bacteriocin of strain *Lactobacilli plantarum*. EDTA functions to diminish the barrier properties provided by the outer LPS membrane of gram negative [72]. Only a few strains within a species produce bacteriocins which give them distinct survival advantages over others. Davey and Richardson [14] conducted a study on purification and some properties of diplococcin from *Streptococcus cremories* and found that only 7% of 150 strains of *L. lactis* subspecies cremoris produced bacteriocins.

Factors limiting the antimicrobial activity of bacteriocins is a valid topic for further research and provides potential benefits to the users of pharmaceutical products as a substitute to conventional antibiotics, to the food industry as a whole. This extensive literature review has critically analyzed the findings of various studies in research articles related to bacteriocins. The review has identified and discussed the factors perceived to be consequential to either activating, inactivating or maintaining the optimal activity of bacteriocins as follows.

2.1. Enzymes

Bacteriocins are proteinaceous in nature. This characteristic has been confirmed by testing their sensitivity to proteolytic such as pepsin, proteinase K, trypsin, chymotrypsin and trypsin and non-proteolytic enzymes such as catalase and α-amylase [46]. In a study by Elayaraja [20], it was reported that when bacteriocin of *L. murinus* AU06 was treated with chymotrypsin, proteinase K, trypsin and pepsin their antimicrobial activity was completely inactivated. Studies have reported that bacteriocins from different species can either be activated, inactivated or do not result in any changes of antimicrobial activity [20,31].

Elayaraja, [20] reported that when bacteriocin of *L. murinus* AU06 is treated with catalase and α-amylase, no changes of antimicrobial activity indicating that antimicrobial inhibition is not due to hydrogen peroxide. This confirms that antimicrobial activity does not require carbohydrate moieties. Todorov et al. [68] and Elayaraja, [20] showed that bacteriocin of *L. plantarum* bacST202Ch and bacST216Ch have shown similar inhibition results by proteinaceous inhibitors. Moreno et al. [6] reported that the activity of bacteriocin can be affected by several factors such as constituents from the cells, interaction with other bacteriocins, growth medium purity and concentration of exogenously added enzymes

In a candid study by Marie et al. [40], they showed that when partially purified bacteriocin Lp6SH was treated by trypsin, α-chymotrypsin and pepsin, complete inactivation was observed. When the same bacteriocin Lp6SH was treated with α-Amylase and lipase, these enzymes did not affect its antimicrobial activity. This suggests that the bacteriocin is not attached to a carbohydrate or lipid moiety. Studies by De Vuyst and Vandamme [15] and Todorov et al. [69] reported similar results for other bacteriocins of *Lb. plantarum*.

The bacteriocin from *L. plantarum* has been reported to be inactivated by papain, pepsin, and lipase enzymes and displays a higher growth reduction potential against *L. ivanovii* compared to that of *P. pentosaceus* which was

sensitive to papain, pepsin, and lipase [69]. However, since in this study the activity of the filtrate was not completely inhibited, there are possibilities that the bacteriocin may also be bound to other molecules like a lipid or a carbohydrate moiety [22,48]. These available data from various reported studies clearly show that the antimicrobial substance such as bacteriocins are of a proteinaceous nature.

Sivakumar & Saif, [62] reported on the effect of enzymes on the inhibitory activity of bacteriocins isolated from *L. acidophilus* and *P. acidilactici* while researching on the partial characterization of Bacteriocins produced by *Lactobacillus acidophilus* and *Pediococcus acidilactici*. In their study α-Amylase and protease had no zones of inhibition while lipase and lysozyme showed zones of inhibition of 6mm on bacteriocins isolated from *L. acidophilus*. These observations were also made in bacteriocins isolated from *P. acidilactici* with the difference in the diameter of the zone of inhibition of lipase and lysozyme reducing to 4mm.

Maina et al. [39] exposed crude bacteriocins from *Bacillus* isolates to proteinase K, trypsin and lipase enzymes, the zones of inhibition reduced significantly. Crude bacteriocins exposed to trypsin lost 40% of their activity while those exposed to proteinase K did not show any zone of inhibition. Nevertheless, lipase lost only 15% of the bacteriocin activity.

Maina et al. [39] concluded that proteolytic enzymes affected the activity of crude bacteriocins because they are proteinaceous in nature. However, the bacteriocins may have a lipid moiety whose hydrolysis by the lipase enzyme does not lead to significant loss of the crude bacteriocins' activity.

Teo & Tan, [66] studied two strains of *B. subtilis*, PB3 and PB6 and observed that they had antimicrobial activity against *C. perfringens* ATCC 13124. They further indicated that filtrates of these *Bacillus* species contained a proteinaceous antimicrobial factor that was stable in the presence of high heat, bile salt and solvents. According to Karthikeyan and Santosh, [33] report, catalase and amylase had no effect on activity bacteriocin produced from *Lactobacillus plantarum* while protease completely inhibited the activity of the bacteriocin compound.

2.2. Temperature

Form the available studies, researchers have revealed that bacteriocins are stable over a broad range of temperatures [56,52]. In a study by Stoffels et al. [64] when analyzing the effects of bacteriocins isolated from *Carnobacterium* sp it was reported that bacteriocins are stable between 30°C – 80°C. They further reported that even at 60oC, bacteriocins retained more than 60% of their activity for 30 minutes and declined subsequently. Various studies have reported that bacteriocins of the two strains of LAB are considered to extremely heat stable. In a study by Campos, [7] the effects of heat was determined from bacteriocins of these two strains of LAB. It was reported that these bacteriocins from LAB can withstand heat treatment after 15 min at 121°C without altering their antibacterial activity.

Fatima and Mebrouk, [22] reported that the storage of the active compounds, bacteriocins at +4°C for three months and in a frozen state -20°C did not affect the antibacterial activity. Scannell et al. [59] reported that full bacteriocin activity can be retained upon storage at +4°C up to 8 months. Bacteriocin Lp6SH has been reported by KR & Tallapragada, [37] to be heat resistant. They found that extraordinarily 80% of their activity could still be recorded against *L. plantarum* strain 3SH after 30 min at 121°C.

When Maina et al. [39] heated crude bacteriocins at different temperatures up to 80°C and tested them against bovine mastitis pathogens, they found that the temperatures below 80°C did not cause any significant reduction in the antimicrobial activity of bacteriocin. However, exposure to temperatures above 100°C - 121°C resulted in a more than 50% fall in antimicrobial activity that was significant. These findings showed that crude bacteriocins from *Bacillus* are effective up to 80°C. These *Bacillus* bacteriocins have been found to be usually thermal stable [65].

Cherif et al. [9] and Cherif et al. [10] studied Entomocin 110 obtained from *B. thuringiensis* sub species *entomocidus* HD110 and entomocin 9 and showed that they retained their activity at 53% and 72% of respectively even after autoclaving. Sharma and Gautam, [60] reported that BLIS bacteriocin from *B. mycoides* was stable at 100°C. Sivakumar & Saif, [62] subjected bacteriocin from *L. acidophilus* and *P. acidilactici* to heat treatments at two different temperatures i.e. 100°C and 121°C and determined the remaining activity using the ADT method after heat treatment. The results showed that the crude bacteriocin could be boiled for 30 minutes without the loss of activity. However, bacteriocin isolated from *L. acidophilus* and *P. acidilactici* were completely inactivated after 10 minutes exposure to 121°C.

Sivakumar & Saif, [62] analyzed the effect of time and temperature of storage on bacteriocin activity Bacteriocins from the two organisms were stored at –20°C, 4°C and 37°C. The results indicated that bacteriocin could be stored at –20°C for at least 45 days and at 4°C for 20 days. However, during storage at 37°C, a significant loss of activity occurred from 5 days. This loss of activity was attributed to the action of proteolytic enzymes which were present in the supernatant fluid. These results were in accordance with the earlier reports [2,45].

Karthikeyan and Santosh, [33], during their isolation and partial characterization of bacteriocin produced from *Lactobacillus plantarum* tested the bacteriocin activity with different temperatures i.e. 10, 20, 30, 40, 50 and 60°C and the activity was found to vary from 1600 to 12800 AU/ml, the maximum arbitrary unit was measured as 12800 AU/ml at 40°C. Todorov et al. [70] during their Partial characterization of bacteriocins produced by three strains of *Lactobacillus sakei*, ST22Ch, ST153Ch and ST154Ch, isolated bacteriocins that were heat tolerant and remained active after 2h at 100 °C.

2.3. pH

Studies have focused and brought to the limelight the effects of the potential of hydrogen on the optimal activity of bacteriocins. Tagg et al. [65] and Ennahar et al. [21] determined this influence of pH and revealed that 100% active stability of bacteriocins was observed between the

pH 6-7 and at pH 4, more than 70% of activity was retained. Bacteriocin Lp6SH was found to be stable in the pH range 2.0 to 10.0 by Marie et al. [40]. This Bacteriocin Lp6SH pH range is broader than the pH range reported in bacrteriocins isolated from other bacteria.

Fatima and Mebrouk, [22] studied the pH stability in the range of pH 2 to 12 and observed that these bactériocins were active at pH values from 2 to 6.0. They further reported that this stability reduced at higher pH levels. In their study, they adjusted the pH to 6.5 to eliminate organic acids. Silva et al. [61] determined the effects of pH bacteriocins of the two strains of LAB are considered to extremely heat stable.

Liu and Hansen, [38] reported that the only bacteriocin commercially used as a food additive in acidic conditions and is unstable in alkaline pH is nisin. Bacillocin 490, a bacteriocin from *B. licheniformis* has been reported to show antibacterial activity between acidic to alkaline pH [41]. In food industries, bacteriocins produced in alkaline conditions are now gaining more attention because pH of many food products is between neutral to alkaline. Sharma and Gautam, [60] in their study of BLIS bacteriocin from *Bacillus mycoides* showed that it was stable over a wide pH range of 4–11.

Maina et al. [39] assayed the ability crude bacteriocins from *Bacillus* isolates to inhibit the growth of *E. coli* ATCC-25922 and *Staphylococcus aureus* ATCC 25923 at a range of pH range between 3 and 9. They reported that no zone of inhibition was observed at pH below 5 against bovine mastitis pathogens. However, their study showed that a mean zone of inhibition of 19.57mm was observed for pH range between 7 and 9. These results further showed that these bacteriocins isolated from the *Bacillus* isolates are affected by acidic pH environments and work well in neutral and alkaline conditions. In the year 2009, Xie and others isolated a bacteriocin from Chinese herbs that were stable at pH range between 3 and 10. Sivakumar & Saif, [62] also observed that bacteriocins are resistant to wide range of pH and temperature. In their study, bacteriocins produced by the isolates were stable at a range of pH 3–9 up to 24h. However, little inactivation occurred only at pH 10 and 11.

Ogunbanwo [45] observed that the activity of bacteriocin produced by L. brevis remained constant after heating at 121°C for 16min and at pH 2-8 but declined thereafter. These results were in accordance with the results published by Sivakumar & Saif, [62]. Similarly bacteriocins from *L. plantarum* remained constant after heating at 121°C for 10 minutes and were stable at pH 2-6 followed by a subsequent decline. The resistance of bacteriocin to a wide range of pH and heat treatment is consistent with the smaller molecular weight of purified bacteriocin. These properties have been reported to resemble those of Thermophilin A and Thermophilin 347 [2].

The studies of Tagg et al. [65] and Davey [14] reported that the action of bacteriocin on susceptible cells require an adsorption on the cell envelope receptors of sensitive microorganisms. According to [2] the adsorption of Thermophilin T to sensitive cells occurrs in the pH range 2-4, with the maximum of 75% at pH 2. In the case, Sivakumar & Saif, [62] adsorption of bacteriocins to sensitive cells occurred in the pH range of 3-4. Nevertheless, inhibitory activity did not require an acidic environment. Bhunia et al. (1991) studied the mode of action of pediocin AcH from *P. acidilactici* H on sensitive cells of *L. plantarum* NCDO 955 and reported that maximum adsorption occurs at pH 6.0 - 6.5.

Jack et al. [29] conducted a study of the effect of various pH values from 1-12 and showed that maximum activity occurs at pH 4 and 5. Regarding pH, Karthikeyan and Santosh, [33] found that the maximum inhibitory activity of bacteriocin produced from *Lactobacillus plantarum* varied from 400 AU/ml to 12800 AU/ml, the maximum arbitrary unit was measured as 12800 AU/ml at pH 5.0. According to Sivakumar & Saif, [62], Lactobacillus and their by-products can be used for several applications. Bacteriocins of *Lactobacillus* and *Pediococcus* are inhibitors of food spoilage pathogens.

2.4. Salts

In a study by Ivanova et al. [28] they reported that partially purified bacteriocin was not sensitive to NaCl, Tween 80, Tween 20 and Triton X-100. However, they found that bacteriocin activity was reduced by SDS and Urea. The antimicrobial activity of the mixture of EDTA and partially bacteriocin was stronger than bacteriocin or EDTA tested alone. Atrih, et al. [4] reported that plantaricin C19 produced by *Lb. plantarum* C19 lost its activity after treatment with SDS or Triton X-100. Karaoğlu et al. [31] examined different concentrations of inorganic salts such as NaCl and KCl (0, 0.5, 1, 3 and 5 % w/v) for their inhibitory effect on bacteriocin preparations and found that there was an increase in bacteriocins activity of the both strains against *L. ivanovii* on increasing the concentration of NaCl and KCl up to 5%.

Fatima and Mebrouk, [22] observed that the best organic solvents for the activity of bacteriocin produced by *L. plantarum* were hexane followed by acetone, where the activity was 50%. In their study, Fatima and Mebrouk, [22] further reported that *P. pentosaceus* activity was stable when hexane, acetone, and 90% alcohol organic solvents were applied. In order to scrutinize the influence of inorganic salts on the activity of the CFSs of the two LABs against the indicator strain used after 2 h of exposure, Fatima and Mebrouk, [22] used different concentrations of NaCl and KCl. The finding showed that the increase in the concentration of NaCl and KCl up to 50% resulted in an increase in bacteriocin activity on the indicator strain.

Todorov et al. [68] observed that bacteriocin activity was highly sensitive to Triton X-114 and urea and moderately resistant to detergents such as SDS, Triton X-100, Tween 80, Tween 20 and ethylene diamine tetra acetic acid and further showed that the possible effect of organic acids can be eliminated by pH adjustment to 6.5. Regarding various salinity (NaCl %), Karthikeyan and Santosh, [33] tested from 0.1 to1.0% NaCl and the activity of bacteriocin produced from *Lactobacillus plantarum* was varied from 3800 AU/ml to 12800 AU/ml, 0.9% was found to be suitable for the bacteriocin production. Todorov et al. [70] reported that the activity of bacteriocins were not affected by treatment with 1% Triton X-100, Tween 20, Tween 80, SDS, NaCl, urea and

EDTA. However, bacteriocins were inactivated in presence of 1% Triton X-114.

2.5. UV light

The effect of UV light on bacteriocin activity has been studied by using bacteriocins from various organisms such as *P. pentosaceus* and *L. plantarum*. Complete destruction of a bacteriocin produced by *P. pentosaceus* was observed after 74 minutes of exposure to UV light. However, after the same period of exposure to UV light, *L. plantarum* bacteriocin remained stable [22].

2.6. Sugar

It has been observed and reported in various studies that the highest bacteriocin activity is obtained when glucose and peptone are varied to 0.25% and 0.5% respectively in the constituted MRS broth [11,47].

3. Conclusion

Bacteriocins differ greatly with respect to their sensitivity to different pH. Studies on the effect of various pH values ranging from 1-12 show that optimal activity is attained at pH 4 and 5. Most bacteriocins produced by lactobacilli are considered to be more tolerant of acid than alkaline pH values. With regard to enzymes, bacteriocins are generally destroyed by trypsin, proteinase K and pronase E treatments. Generally, Gram negative bacteria have been reported to be usually resistant to most bacteriocins of *Lactobacillus* strains. Sensibility to proteolytic enzymes evidences the proteinaceous characteristic of bacteriocins.

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