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Isolation, Purification and Commercial Operation of LAB Bacteriocins

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ABSTRACT

A large variety over Gram positive bacteria or Gram negative bacteria in the course of their growth, produce components of protein structure (either proteins or polypeptides) possessing antimicrobial activities, known as bacteriocins. Although bacteriocins are different as compare to antibiotics. Bacteriocins involve a tremendous group of ribosomally combined peptides that as a rule demonstrate antimicrobial activity, these are formed during the primary phase of bacterial growth. They are classified into three classes. Among gram positive bacteria more specifically *lactobacilli* gained more attention due to the production of bacteriocin. These substances are used in the Food industry as characteristic additives. Most of the bacteriocins are bactericidal or bacteriostatic in nature. Different method and techniques are used for the isolation and purification of bacteriocins. Biotechnological procedures including salting out, solvent extraction, ultrafiltration, adsorption-desortion, ion-exchange, and size exclusion chromatography are among the most usual methods. These days, bacteriocins have been broadly used in Food preservation. Nisin is commercially available bacteriocin used for food conservation in spite of a huge number of bacteriocins. Bio preservation is achieved by using nonpathogenic microorganism and their metabolites. Along with bio preservation bacteriocins have been utilized for shelf life extension, clinical antimicrobial action and control of fermentation microflora. Bacteriocins utilized in food industry specifically for different dairy products such as milk, yogurt and cheese. It activity has been widely examined in different products like meat and vegetables. Nisin is the main bacteriocin that has been formally utilized in the food industry and its utilization has been endorsed around the world.

Keywords: Bacteriocins, LAB, Isolation, Food Conservation

I. INTRODUCTION

In microbial biological communities, a few microorganisms incorporate antimicrobial properties, for example, bacteriocins, that obliterate or repress the development of different microorganisms.[1] It has been recommended that the greater part to every single bacterial specie orchestrate bacteriocins.[2] Bacteriocins involve a tremendous group of ribosomally combined peptides that as a rule demonstrate antimicrobial movement to strains that are firmly identified with the producer strain (limit range bioactivity) and now and again to strains crosswise over genera. [3] Bacteriocins can be considered as anti-toxins, they vary from traditional anti-microbial in various viewpoints. [4] Bacteriocins differ from traditional antibiotics as these are protein in nature. Bacteriocins are rapidly destroyed by the action of proteases and degraded in the colon. Bacteriocins are resulted from by means of each Gram-positive and Gram-negative bacteria and the bacteriocins beside Gram-positive bacteria seem after appropriate a broader length of susceptible organisms. Antibiotics are formed as a result of secondary metabolites and inhibitory in action. Bacteriocins range beyond antibiotics concerning the groundwork about theirs molecular size, antimicrobial spectrum, producing species, permanency and bodily properties. Antibiotics utilizes is limited into food and feed industry due to the fact bacteriocins play an important role within it respect. The distinction of antibiotics or bacteriocins are depicted between Table 1. As because of bacteriocin studies, the colicins observed in *Escherichia coli* were the strongest bacteriocins for years. [5] Bacteriocins from many bacteria active against different human and animal microbial pathogens, including methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant enterococci (VRE) without showing toxicity.

Properties of Bacteriocins

Bacteriocins are intrinsically tolerant to higher warm stress and are more dynamic at a more extensive pH go than traditionally anti-infection agents. These antimicrobial peptides are colorless, odorless, yet tasteless, making them best for utilizes as much food preservatives. Advancement of safe strains among their objective microscopic organisms is far-fetched as they have quick acting antimicrobial systems that are exceedingly strong even at low fixations. Besides, their proteinaceous nature limits resistance improvement as they are effectively debased by proteolytic compounds, consequently diminishing the odds of target strains building up any resistance system. Maybe the most encouraging favorable position of bacteriocins over customary anti-microbial is their essential metabolite nature that makes them effectively subjected to bioengineering to either build their bioactivity or to determine their objective microorganisms. [4]

LAB create bacteriocins that offer the possibility to give leeway in rivalry and colonization of the gastrointestinal tract. Lab are gram positive cocci or rods, facultative anaerobes utilized carbohydrates for the production of lactic acid. Mostly they are found in milk, yogurt and other dairy products such as cheese and also in fermented vegetables. They are used in the food industry on the point of characteristic additives. They have been used as a characteristic boundary for microorganism and nourishment waste brought about by bacterial operators has been ended up being productive. Bacteriocins can tolerate high temperature and can perform their actions at different pH (wide pH range). Bacteriocins mainly target the cell wall and pore formation occur. Properties of bacteriocins shown in Fig 1. Most of the bacteriocins are bactericidal or bacteriostatic in nature. The main driving force in the mechanism of action of bacterions, is generally electrostatic action between the bacteriocins and the target cell envelop. They are produce as a result of primary metabolites or secondary metabolites this information is not known yet. Primary metabolites are produced in response of log or exponential phase when there is great quantity of nutrients are available. Secondary metabolites are formed during deceleration phase and they also play vital role in the life of a microorganism. Different lactic acid bacteria produce different bacteriocins such as lactacin F, lactocin 705, lactoccin G, and Naisin produce by L. *johnsonni spp*, L. *casei spp*, and L. *lactic spp* and Lactococcus *lactic spp*. respectively. A third type of Bacteriocins known as Nisin Q has been reported, it was isolated from *lactococcus lactis*. It structurally resemble with Nisin A and Nisin Z, difference occur on only few amino acids positions. The activity of different bacteriocins is not constant and uniform, produced by different lactic acid bacteria. It mainly depends upon the chemical and physical condition of food. The pH is an important consideration in this respect. The lactic acid bacteria have been used since many years, their safety is never been questioned and report of harmful effects have been reported rarely. Resistance may develop in bacteriocins as a result of mutation, certain changes in cell wall or cell membrane. It also occurs due to alteration in fluidly part of the cell, lipid concentration in cell or the thickness of cell may result in resistance. Accurate or proper mechanism of resistance action is still unknown, that how it happens.

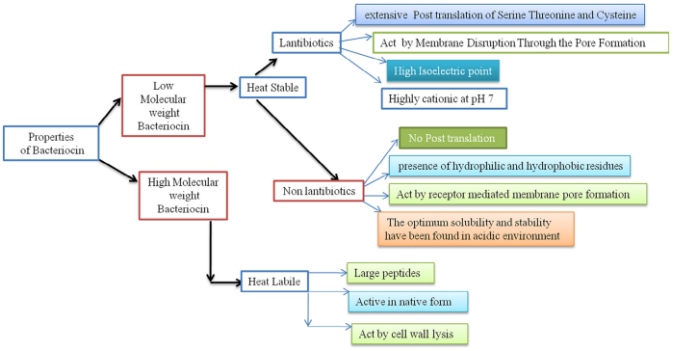

Figure 1. Different properties of Bacteriocins

Mechanism of action of bacteriocins

Bacteriocins exert their action by breakdown regarding the integrity over the cell wall or prohibition over protein and nucleic acid synthesis by means of numerous mechanisms in opposition to Gram-positive or Gram-negative bacteria. Bacteriocins indenture in conformity with cell wall components through specific or non-specific receptor binding, along with lipid or surface molecular binding sites. This apprehension reasons pore form then direct cell lysis, ensuing into cell death with the aid of dissipation over the proton motive force over the bacterial system. [6] Membrane pore formation mechanism has also been discovered in bacteriocin colicin against Gram-negative bacteria. The barrel stave model, the carpet model and the wedge model have also

been proposed in support of pore formation. The mechanism of action of bacteriocin is shown in Fig 2.

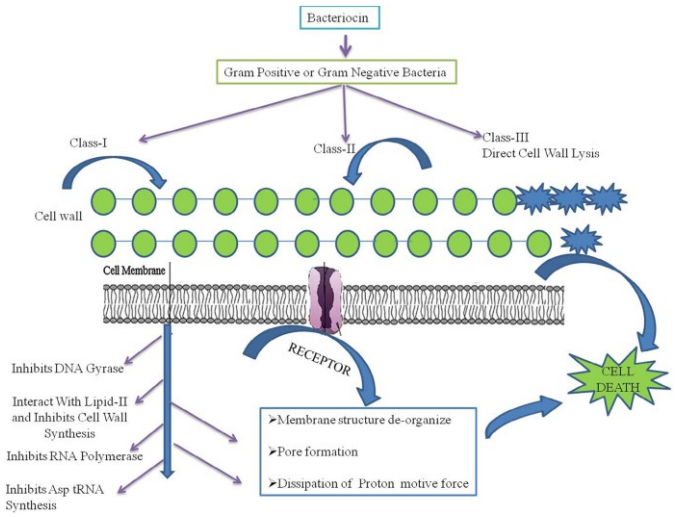

Figure 2. Mechanism of action of bacteriocins

Classification of LAB Bacteriocins

Throughout the years, different order plans of LAB bacteriocins have been recommended.[2] The characterization conspire proposed by Cotter and colleagues (2005) is the most broadly acknowledged, constraining the gathering in one of two classes, one is the class I (lantibiotics) the other is class II (non-lantibiotics-containing bacteriocins). The most eminent change in this new plan is the recommended rejection of class III bacteriocins and renaming them as bacteriolysins since they are lytic proteins as opposed to peptides. As of late, Heng and colleagues (2007), despite the fact that concurring comprehensively with this arrangement plot, recommended a further change in which round bacteriocins ought to be assembled into an alternate class. [7]

The vast majority of bacteriocins are positively charged, warm steady having hydrophilic and hydrophobic portions. These are usually pass through the membranes. These are classified in three noteworthy classes and their order had been continually amended all through the most recent decade because of the broad research acknowledged. [8] Bacteriocins generally show little adsorption specificity. Gram positive microorganism permits section of generally expansive atoms. At low pH values bacteriocins have more noteworthy antibacterial action. Gram positive (+) microscopic organisms as well as the transporting cells are pH subordinate. Lipoteichoic acid and teichoic acids are the negatively charge surface polymers which, are essential in underlying connection of bacteriocins created by positive class microorganisms. Inside all classes of bacteriocin amino corrosive succession homologies present not only inside the develop peptide, but also in the terminal-N pioneer area related to bacteriocin proteins discharge and handling.[9] It has become obvious that bacteriocins are heterogeneous group of compound after the discovery of new bacteriocins. Nisin is commercially available bacteriocin used for food conservation in spite of a huge number of bacteriocins. This is widely used against highly pathogenic microorganism such as *Staphlococcus aureus* and *Listeria monocytogense* and prevent from food spoilage. In 1988 FDA has approved it against chill products such as cheese spread, pasteurized cheese and heat treated soups. Nisin has pentacyclic structure and composed of 34 amino acids. It has four β-methyllanthionine residues and one lanthionine residue. It has great ability against high temperature, can withstand a high temperature of 121^{o}C. The processes include in the formation of nisin is transcription, transduction and post transduction modifications.

Class I

The lantibiotics encloses the trademark polycyclic thioether which acts on cell membrane and transformed into lanthionine or methyllanthionine amino acids and in addition the dehydroalanine and 2-aminoisobutyric corrosive unsaturated amino acids are involved. These are can be formulated further into two sorts in light of auxiliary similitudes. Nisin is a much known example in this category. The composition of nisin shows it contains total 34 amino acid which are covalently attached with each other. The use of nisin is safer, it does not cause any harmful effects on human health. So, it is largely used in food industry in this respect. Sort A involves moderately lengthened, screw formed, emphatically charged, amphipathic, adaptable atoms. Higher concentration of nisin is active against gram negative rods as well. It was earlier divided into two subclasses. Their atomic mass differs between 2 to 4 kDa and they for the most part act as a consequence of orifice development. There is loss of membrane polarization in the intraplasmic layer of the touchy mark species. The nisin and lacticin 3147 are the actual delegates. Sort B bacteriocins are in cylindrical form meddle with the cell chemical responses. The atomic size lies in two to Three

kDa which possibly show that these molecules having a total negative site or no charge site. [1]

Class II:

Bacteriocins of Class II origin are additionally little having size of less than 10 kDa, moderately heat stable in nature. These bacteriocins are heterodimer in structure. Non-lanthionine contains film dynamic peptides, which are isolated and sub classified in two. Pediocin like or listeria like dynamic bacteriocins are exist in subclass II, while other subclass has a terminal-N accord succession of Tyr-Gly-Asn-Gly-Val-Xaa-Cys sequence. A great level of similarity approximately (40%-60%) is demonstrated, when the comparing amino corrosive successions are adjusted. They are blended with a forge peptide which should be evacuated by proteolytic preparing. Most of the part after a dilution gives glycine deposit for instance like sakacin A and pediocin -1. [10] Subclass II bacteriocins alludes two independent peptide which implies the requirement of two peptides to act harmoniously keeping that in mind the end goal is to have an antibacterial movement, and lactococcin G and Lactacin F are individuals from this gathering.[11]

Class III

This class is comprised of warmth, labile proteins which are having sub atomic weight of >30 kDa, and this class has not been broadly explored. Class III bacteriocins are helveticin I which are created by *L. helveticus* and *Enterococcus faecium*, delivered enterolysin. [12]

Extraction and Purification

Lactic acid microbes screening from various sources that can fluctuate in plant material and food items for human or creature disconnects is the initial step for segregation of bacteriocins. Moreno in 1999 have detached a few species of lactic microorganisms for the nourishment items such as milk and cheddar. [13] Through the well dispersion measure on agar plates the hostile action was recognized.[14] Catalase was added to the way of life medium, to keep away from threat by hydrogen peroxide, the strong medium is achieved by the addition of phosphate support to bar restraint by natural acids. The assurance of insignificant inhibitory fixation is permitted by extra tests in fluid medium which are corroborative. The sanitization of bacteriocins uses different systems from complex development juices have abused their negatively non polar attributes.[15] Normal techniques for bacteriocins isolation depend on their fondness of natural solvents. At a given pH esteem their variety in dissolvability occur in concentrated salt arrangements. The Bacteriocins nearness of hydrophobic districts is fundamental for their movement against touchy microscopic organisms. Since the hydrophobic cooperation between the bacterial cells and bacteriocin atoms relies on upon the inactivation of microorganisms by bacteriocins.[16] Sub-atomic mass of some bacteriocins show up in their local condition as totals high (ca. 30–300 kDa). These totals can veil in part or totally the antibacterial movement of the bacteriocins, amid their filtration and furthermore instigate mistakes in assurance of their sub-atomic size. That is particularly valid with very non polar, low sub atomic weight bacteriocins. This is effortlessly connected with asserted cells of extracellular material (e.g., cell divider flotsam and jetsam and lipotheicoic acids micelles) and other hydrophobic mixes from the way of life condition.[17] In every one of these cases high atomic weight molecules edifices might be desegregated by utilizing separating operators, like SDA and urea.[18] As per their size as well as physicochemical properties the bacteriocins are recouped from the sans cell supernatants, and they can be focused by procedures allowing partition of the portions.[17]

Purification Techniques

Bacteriocin production can be achieved by batch fermentation, which generally depend upon composite media and well established physical conditions. [19] It suggests that pH and temperature are important factors in it. De Vuyst and Vandamme in 1992 proposed that bacteriocin generation is associated with bacterial development. [20] It suggested that the volumetric bacteriocin creation is subject to the aggregate biomass arrangement. In the wake of coming to a maximal bacteriocin movement in the aging medium amid the dynamic development stage, frequently an exceptional decline in dissolvable bacteriocin action happens. The bacteriocin disappearance activity action was attributed to proteolytic inactivation protein aggregation. Bacteriocin molecules adsorption occur to the cell surface of the producer cells.[20] The aggregation can be prevented by adding ethanol to the fermentation medium.[21] Fed-batch fermentation technology also use for production of bacteriocins. It permits the acquiring

of high cell densities through the continuous supply of fresh medium. The expansion rate can be monitored by the use of development constraining sustaining procedures. Most procedures begin with a stage to think bacteriocins from the way of life supernatant, utilizing for instance diatomite calcium silicate, since bacteriocins are emitted into the way of life medium, or ammonium sulfate precipitation. [22, 23] there are different bacterial culture and media are used for the production of Bacteriocins by using different bacterial strains. For this purpose different volume fermenters are being utilized on smaller or larger scales by using certain composite media. For example MRS media containing 25 % glycerol is used for the preparation of inoculum. The pH adjusted to 5.0 of MRS by adding different concentration of glucose and nitrogen sources. The transfer of nutrient is maintained aseptically and sterilization is an important consideration. Growth is ensured by maintain suitable conditions such as pH, temperature and continuous supply of nutrients to ensure the maximal production of biomass. In spite of the fact that these methods are utilized basically to diminish the working volume, they don't give a high level of decontamination. [24] In Fed batch culture continuity of nutrients is important. Hence, ensuing strides by utilizing preparative isoelectric centering as well as different chromatographic partitions which includes gel filtration, cation trade, hydrophobic connection and turn around stage fluid chromatography are important to accomplish critical sanitization of bacteriocins. As a rule, yet not generally, the yields acquired are low. This is most likely because of the high number of ventures in the convention, prompting tedious procedures and along these lines low yields. To decide ideal parameters for the bacteriocin creation, it is important to decide the perfect states of development of the lactic strains and the piece of the way of life medium. Nature of supplements for ideal generation of bacteriocins, the medium generally contains a multifaceted, however high substance of peptides may meddle in the filtration procedure.[25] A perfect convention for bacteriocin creation ought to be one that is material to vast scale purging, prompting bacteriocin yields higher than half and immaculateness around 90%.[26] The development in an appropriate fluid supplement medium under ideal conditions for bacteriocin generation, As a rule for the non-lantibiotic bacteriocins, the techniques include evacuation of the cells took after by fractionated precipitation of the proteins from the way of life supernatant by expansion of ammonium sulfate. The hastened proteins are in this manner broke down in deionized water or in a frail cushion, and bacteriocin particles are isolated by utilization of various strategies including hydrophobic, particle trade, and size avoidance chromatography. In spite of the fact that these methods have encouraged creation of very cleaned bacteriocin arrangements, the last yield has for the most part been underneath 20% and includes a few days of handling.[27] Numerous lactobacilli species have been distinguished as makers of bacteriocins. The cause of these species has basically been from dairy and vegetable maturations, vacuum-bundled meat items and from creature or human segregates. [25) Purging of bacteriocins was made by utilizing ammonium sulfate precipitation, particle trade chromatography, hydrophobic collaboration and invert stage HPLC. The microscopic organisms having a place with the class Carnobacterium were at first perceived as nonaciduric lactobacilli. These microorganisms are presently separated from Lactobacillus by their failure to develop on acetic acid derivation agar at pH 5.6, their capacity to grow at high pH (8.5-9.5) and their capacity to create L-lactate and oleic corrosive. Bacteriocins creating strains were disengaged from poultry, fish and vacuum-bundled meat. Among the carnobacteria, carnocin, carnobacteriocins BMl and B2 have been filtered.[25] A bacteriocin-like substance created via Carnobacterium pscicicola L103 was somewhat refined by ammonium sulfate precipitation and gel filtration on Sephadex G-25, trailed by lyophilization, keeping in mind the end goal to test its movement against Listeria monocytogenes in vacuum-bundled meat.[24]

Commercial Operation of Bacteriocins

These days, bacteriocins have been broadly used particularly in the field of food preservation industry. The utilization of bacteriocins in food industry particularly on dairy, egg, vegetable and meat items has been widely examined. Among the LAB bacteriocins nisin A and its normal variation nisin Z has been turned out to be exceedingly successful against microbial operators bringing on food harming and waste. Moreover nisin is the main bacteriocin that has been formally utilized in the nourishment business and its utilization has been endorsed around the world. [27] Various safeguarding strategies however, have been utilized as a part of request to anticipate food harming

and deterioration. These strategies incorporate warm treatment (purification, warming disinfection), pH and water action diminishment (fermentation, parchedness) and expansion of additives (anti-infection agents, natural mixes, for example, propionate, sorbate, benzoate, lactate, and acetic acid derivation). Despite the fact that these techniques have been ended up being exceptionally fruitful, there is an expanding interest for normal, microbiologically safe items furnishing the purchasers with high medical advantages. [7] Bacteriocins can be connected on a cleansed or on a rough shape or using an item already aged with a bacteriocin creating strain as a fixing in food handling or consolidated through a bacteriocin delivering strain (starter culture). The consolidation of a bacteriocin creating strain has the detriment of the absence of similarity between the bacteriocin delivering strain and alternate societies required for maturation.[9] In any case, it has been demonstrated that a bacteriocin alone in a food is not prone to guarantee finish security; particularly on account of Gram negative (-) microscopic organisms this has been evident. At that point the utilization of bacteriocins must be consolidated with different advancements that can upset the cell film so bacteriocins can kill the pathogenic microorganisms. [8] For illustration the utilization of non-warm medicines, for example, beat electric field (PEF) is profitable as it doesn't have any impact on food usefulness and wholesome qualities. This method may not be fiscally practical when utilized alone, but rather in lower levels and joined with different medications, for example, bacteriocins might be exceptionally powerful. Moreover bacteriocins could be joined with other antimicrobial mixes, for example, sodium acetic acid derivation and sodium lactate bringing about upgraded inactivation of microorganisms. Bacteriocins can likewise be utilized to enhance food quality and tactile properties, for instance expanding the rate of proteolysis or in the counteractive action of gas blowing imperfection in cheddar. Another use of bacteriocins is bioactive bundling, a procedure that can shield the nourishment from outer contaminants. For example the waste of refrigerated nourishment generally starts with microbial development at first glance that fortifies the alluring utilization of bacteriocins being utilized as a part of conjunction with bundling to enhance food security and self-life.[8] Bioactive bundling can be set up by straightforwardly immobilizing the bacteriocin to the food bundling or by expansion of a sachet containing the bacteriocin into the bundled nourishment, which will be discharged amid capacity of the food item. The steady arrival of bacteriocins from a bundling film on the nourishment surface may have preference over plunging and splashing foods with bacteriocins, in light of the fact that antimicrobial action might be lost or diminished because of inactivation of the bacteriocins by food parts or weakening beneath dynamic focus because of movement into the nourishments. [8] Nisin also used in veterinary industry as a preventive measure of bovine mastitis. Bovine mastitis is a problematic issue not only in japan but also worldwide dairy industry. Injectable nisin are available which helps in cure of bovine mastitis. There are a few strategies to plan bundling movies with bacteriocins. One technique is to fuse bacteriocin specifically into polymers for instance fuse of nisin into biodegradable protein movies. The consolidation of nisin or whatever other bacteriocin can be accomplished through warmth press and throwing into movies produced using soy proteins or corn zein. Another technique is to coat or adsorb bacteriocins to polymer surfaces; cases incorporate nisin methylcellulose coatings for polyethylene movies for the utilization on poultry meat, adsorption of nisin on polyethylene, ethylene, vinyl acetic acid derivation, polypropylene, polyamide, polyester acrylics and polyvinyl chloride.[29] Different application of bacteriocins in pharmaceutical industry have been listed in Table 2.

Table 1: Difference between bacteriocins and antibiotics

Factor Considered	**Antibiotics**	**Bacteriocins**
Production	Secondary metabolite	Ribosomal
Uses	Clinical use	Use in Food industry and clinical
Intensity of bioactivity	Micro to milli molar concentration is r required	Nano to micro molar concentration is required.
Bioactivity spectrum	Broad spectrum activity	Narrow spectrum activity
Thermal stability	Low thermal stability	High thermal stability
Enzyme degradability by proteases	Moderate to high	High
Taste	Present	Absent
Mechanism of action	Active compound are formed by inactivity of genetically transferable determinant	Cell membrane composition adaptation occur through changes
Bioengineering	No	Yes
Mode of action	Intercellular targets or cell membranes involved	Inhibition of cell wall biosynthesis by pore formation on cell membrane

Table 2: Bacteriocins application in pharmaceutical industry

Bacteriocins	Applications in Pharmaceutical
Lantibiotics	It is used in blood pressure treatment. Inflammations and allergies treatment. Skin infections treatment. Mastitis infections treatment. Herpes treatment, Dental carries treatment and peptic ulcer treatment
Colicins	Urogenital infection. Hemorrhagic colitis treatment. Hemolytic uremic syndrome treatment (HUS).
Microcins	Act as an antibacterial agent for the Salmonellosis treatment.

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