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Bacteriocins: Perspectives for Novel and Safest Strategy for Food Preservation

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ABSTRACT: A variety of bacteriocins produced by bacteria that possess the ability of killing or inhibiting the growth of other bacteria have been used in innumerable ways for various applications since last few decades. Bacteriocins being ribosomally-synthesized peptides with antimicrobial activity are becoming a major source in their utilization of food preservation in particular. Potential applications of bacteriocins in food industry can intensively be useful in gradual reduction of use of conventional methods such as addition of chemical preservatives; intensive heat treatments and dehydration thereby making the food which is more naturally preserved and relatively richer in organoleptic and nutrition content. Antibacterial metabolites of lactic acid bacteria and *Bacillus* species possess the ability to be used as natural preservatives thereby hampering the growth of pathogenic bacteria in food. In this review article, various prospects of food preservation by harnessing the antimicrobial activity of bacteriocins have been focused. Thermo stability, wider range of pH tolerance and proteolytic activity has made the utilization of bacteriocin in various preservation aspects to scalable level and commercial purpose.

KEYWORDS*:* Proteolytic; Preservation; Thermostability; Bacteriocin.

I. INTRODUCTION

Preservation is the technique of preventing food from being spoiled either due to microbial growth or the degradation of its own components causing rancidity. From ancient time, people have been using various techniques of preserving food as it is the most susceptible for degradation. A wide variety of natural and chemical preservatives are used even till date. Various theories have also been applied to protect the food from getting spoiled. There are two reasons to why the preservatives should be used anyway. First being the fact that most of the food will get spoiled if there are no preservatives as people now days have shifted to the packed foods and it becomes the necessity of the hour to have some preservatives which not only protect the food but also should be natural and should have no side effects. Other reason is that many people have suffered from major food poisoning and in worst case also have died due to spoillage of food. The center for disease control and prevention in the US reported that there are 76 million cases of food-borne illness each year which results in about 5000 deaths [5][6]. Amongst several biopreservatives present; bateriocins have been considered of prime significance owing to its antimicrobial property against pathogenic bacteria and its utility as natural food biopreservative. Preservation of food without losing the nutrient content is the main concern while making a choice for preservatives. Nevertheless; biopreservatives with the least side effects and ability to maintain intactness of nutritional value of food is the preferred over its existing counterparts. There has been substantial increment in demands of processed and packaged foods globally. Increasing shelf life of the food and keeping the product quality intact with negligible loss in nutritive content is the major issue to be addressed. Different technologies for preservation have been devised that prolong freshness and safety of food thereby ensuring stability in food supply. Several methodologies include use of chemicals [e.g. hydrogen peroxide, organic acids, etc.][1], antimicrobial compounds and many physical methods which includes heat preservation, vacuum preservation and many more. Precisely, preservatives are divided into three types; Natural preservatives, Chemical preservatives and Microbial preservatives. The natural preservatives are the ones which are harmless and include salt, sugar etc. Chemical ones are mainly anti-microbial compounds or anti-oxidants which involves inhibitory effect on growth of the microorganisms. Lastly, microbial preservatives inhibit growth using compounds which are being synthesized by microbes themselves [2]. Bacteriocins have attained an utmost importance in the field of food preservation due to its antimicrobial properties and also because some of them have got recognition as GRAS [generally regarded as safe] by FDA] e.g. Nisin [3][4]. The paper discuss about different categories of preservatives with a special focus on bacteriocins discussed with brief introduction, their mode of action, with special preference to nisin.

II. CHEMICAL PRESERVATION & FOOD IRRADIATION

Chemical food preservatives refer to substances which, under certain conditions, either inhibit or delay the growth of microorganisms without necessarily destroying nutritional content. Some natural food constituents also may act as preservatives which, when added to foods, retard or prevent the growth of microorganisms for example use of Sugar in making jams, jellies, and marmalades and in candying fruit ; Use of vinegar and salt in pickling and of alcohol in brandying . Some chemicals that are added foreign to foods also prevent the growth of microorganisms. The chemical preservatives that are naturally present include antioxidants e.g. Vitamin C, compounds e.g. BHA, and bacterial growth inhibitors e.g. organic acids etc. [7]. Sodium benzoate and other benzoates in prescribed quantity [usually not exceeding 0.1 percent] are among the principal chemical preservatives. Benzoic acid is more effective against yeasts than against molds and bacteria. Also; vanillic acid esters, monochloroacetic acid, propionates, sorbic acid, dehydroacetic acid, and glycols may be used as chemical preservatives. Also, Sulfur dioxide and sulfites are amongst the widely used inorganic chemical preservatives. Sulfites are more effective against molds than against yeasts and are widely used in the preservation of fruits and vegetables. Sulfur compounds are extensively used in wine making and, as in most other instances when this preservative is used, much care has to be exercised to keep the concentrations low in order to avoid undesirable effects on flavor. Oxidizing agents such as nitrates and nitrites are commonly used in the curing of meats. Choice of chemical substances to be used as preservatives depend upon the type of food and longevity required for preservation. Chemical preservatives which are commonly used by food industries in preservations are categorized as Weak acids: - which include acetic acid, lactic acid, benzoic acid and sorbic acids. Mode of action of these compounds lies in mechanism called pH dependent equilibrium between dissociated and undissociated state of any acid. Although certain gram positive bacteria have got some resistance as the mechanism involves penetrating in bacterial membrane and causing ionic imbalance. Certain bacteria which are destined to live in the low pH conditions for e.g., pseudomonas species that reside in human stomach where the pH is already three are not affected by these mild acids. Some of the microbes like yeasts are also known to possess induction of a membrane integral protein hsp30 which also provides certain tolerance. Other category includes comparatively strong acids used as preservatives that include hydrogen peroxide which aims at DNA damage of microbes and also of some spores. Resistance mechanisms have been developed which protect the population by activating enzyme catalase that neutralizes the effect of hydrogen peroxide. On the other side Spores are resisted by the presence of some small acid- soluble proteins of a/b type that are synthesised in the developing spores which protect DNA in the dormant spore. Chelators may also be used in accordance to other chemical preservatives which act as membrane permeabilising agents. Chelators like EDTA and others aid in the potential of other chemical preservatives to affect the growth of gram negative bacteria [1].The actions of mainly all chemical preservatives are based on membrane composition and potential by which different chemicals are entering in the cell and are either destroying DNA of microbe or causing disruption of the membrane. However varieties of microbes have developed a defense or a resistance mechanism for a lot of chemical preservatives for e.g. yeast has developed a vast array of defense against antimicrobial compounds [9]. The chemical preservatives like sulphites, nitrites, benzoates, sorbic acid etc. are known to cause the variety of human health disorders like utricularia, dermatitis etc. Chemical preservatives are also known to cause the allergic reactions in some people and that too after 2-3 days of consumption. These allergic reactions takes place as they mix with blood and triggers allergy that is why these compounds are also known as trigger allergens [7]. Alternatively use of either high-speed electron beams or high-energy radiation with wavelengths smaller than 200 nanometres, or 2000 angstroms (*e.g.*, X rays and gamma rays) is used as the preservatives. These rays contain sufficient energy to break chemical bonds and ionize molecules. The two most common sources of high-energy radiation used in the food industry are cobalt-60 (^{60}Co) and cesium-137 (^{137}Cs). For the same level of energy, gamma rays have a greater penetrating power into foods than high-speed electrons. Earlier, people believed that radiations may cause some health problems to humans as well but research showed that the radiations may change the color of food but not its quality [7][8].

III. BIOLOGICAL OR MICROBIAL PRESERVATIVES

Chemical preservatives no doubt provide microbial destruction to an extent but also have the disadvantages of posing a threat to human health. The ever increasing concern for health has led researchers to devise a new way of biopreservation. Biopreservation systems in foods seem promising for food industry with least side effects.

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Bacteriocinogenic lactic acid bacteria and their isolated bacteriocins are considered safe additives that are useful control the frequent development of pathogens and spoiling microorganisms in foods. Demand for products with fewer chemicals thereby seems to create the necessity of exploring new alternatives, in order to reduce the side effects of chemicals. The microbes have various defense mechanisms by which it is able to sustain its viability, which include broad spectrum antibiotics [secondary metabolite of many microorganisms to kill other species], or the lytic enzymes like lysozymes found in many types of food products, metabolic by products like lactic acids of lactobacillus, numerous types of protein enterotoxins and the bacteriocins released by bacteria which are known to have bactericidal mode of action [10] over the closely related species. Ever since the beginning of era, bacteria and other microbes like yeast, fungi etc. have been used in food products and have found an extensive use in fermentation and related products. Lactobacillus has been used in the fermented food products due to their beneficial influence on nutritional, organoleptic, and shelf-life characteristics. They cause rapid acidification of the raw material through the production of organic acids, mainly lactic acid [11]. In this context, bacteriocins are indicated to prevent the growth of undesirable bacteria in a food-grade and more natural way, which is convenient for health and accepted by the community. According to their properties, structure, molecular weight [MW], and antimicrobial spectrum, Bacteriocins are classified in three different groups: lantibiotics and non-lantibiotics of low MW, and those of higher MW. Several strategies for isolation and purification of bacteriocins from complex cultivation broths to final products have been proposed. Biotechnological procedures including salting-out, solvent extraction, ultrafiltration, adsorption-desortion, ion-exchange, and size exclusion chromatography are among the most usual methods. Peptide structure-function studies of bacteriocins and bacterial genetic advances make it easier to understand the molecular basis of their specificity and mode of action. Nisin has been proved of commercial success, as a good perspective and new avenues of research are open to continue the study and development of new bacteriocins and their biotechnological applications. These substances in appropriate concentrations may be used in veterinary medicine and as animal growth promoter instead usual antibiotics, as well as an additional hurdle factor for increasing the shelf life of minimal processed foods.

IV. BACTERIOCINS

Bacteriocins are ribosomally synthesized peptides originally defined as proteinaceous compound that affects the growth and viability of closely related micro organisms [18]. Bacteriocins may serve as anti-competitor compounds enabling the invasion of a specie or a strain in an established culture [15][16][17] or may act as a communication molecule in a microbial consortia like biofilms [14]. Both the gram positive and gram negative bacteria have the ability to produce bacteriocins [13]. Among the gram positive bacteria, especially lactic acid bacteria, lactobacilli have gained particular importance in the field of production of bacteriocins. Nisin is the best known bacteriocins of LAB which has been used for more than 30 years in foods safely [19]. Among the gram negative bacteria, colicins are produced by E.coli. Research is going on extensively to explore the nascent field of biopreservation. Isolation of different types of bacteriocin producing strains and characterization of bacteriocin produced by them for food preservation is prevelant. Many lactic acid bacteria [LAB] produce a high diversity of different bacteriocins. Though these bacteriocins are produced by LAB found in numerous fermented and non-fermented foods, nisin is currently the only bacteriocin widely used as a food preservative. Many bacteriocins have been characterized biochemically and genetically, and though there is a basic understanding of their structure-function, biosynthesis, and mode of action, many aspects of these compounds are still unknown. Colicin was the first identified bacteriocin. Colicin gene clusters are encoded on plasmids which have three genes; the colicin gene, the immunity gene, and the lysis gene [10]. The similar kinds of molecules are secreted by other organisms as well such as killer toxins of yeast, defensins of mammals, cecropins of insects, tachyplesins of crabs, magainins of amphibia,thionins of plants and many more.

Bacteriocins are regularly been confused with antibiotics but they are different in having broad range spectrum. Also Cleveland and others in 2001 said that antibiotics can elicit allergic reactions in humans, whereas, bacteriocins are degraded in the human gut by the enzyme trypsin. In addition, antibiotics are the secondary metabolites but bacteriocins are produced in the log phase [19]. Due to safety, a broad range of bacteriocins have been identified and are used in food items.

V. CLASSIFICATION OF BACTERIOCINS OF LAB

The major classes of bacteriocins produced by LAB include: [I] lantibiotics, [II] small heat stable peptides, [III] large heat labile proteins, and [IV] complex proteins whose activity requires the association of carbohydrate or lipid moieties [20]. Class I comprise of bacteriocins which contain lanthione containing peptides. They are the small peptides and differentiates from others as it contains didehydroamino acids and thio-ether amino acids i.e. lanthione and 3-methyl lanthione [21][22]. It has two subgroups; type A and type B; on the basis of their distinctive ring structure. Type A are the screw shaped amphipathic molecules with two to seven positive charge whereas type B are the globular one with either no net charge or net negative charge [23]. Class II are the small, relatively heat stable and non lanthione containing active peptides which are subdivided into 3 categories; i.e., class II a , class II b and class II c on the basis of following: N terminal sequence, formation of bi component pores, and the presence of functional sulfhydryl groupings. Class III is the large heat labile proteins with large molecular weight example, Helveticin I produced by *lactobacillus helveticus* and enterolysin produced by *Enterococcus faecium* [25]. Class IV is the complex bacteriocins that contain essential carbohydrate or lipid moieties in addition to protein [23]. However, the inhibitory activity of these substances is confined to Gram-positive bacteria as inhibition of Gram-negatives by these bacteriocins has not been demonstrated. Cytoplasmic membrane which forms the border between the cytoplasm and the external environment is surrounded by a layer of peptidoglycan layer which is significantly thinner in Gram-negative bacteria than in Gram-positive bacteria. Gram-negative bacteria possess an additional layer, the so-called outer membrane which is composed of phospholipids, proteins and lipopolysaccharides [LPS], and this membrane is impermeable to most molecules. Nevertheless, the presence of porins in this layer will allow the free diffusion of molecules with a molecular mass below 600 Da. The smallest bacteriocins produced by lactic acid bacteria are approximately 3 kDa and are thus too large to reach their target, the cytoplasmic membrane [20]. However, Stevens et al. [1991] and Ray [1993] have demonstrated that Salmonella species and other Gram-negative bacteria become sensitive to nisin after exposure to treatments that change the permeability barrier properties of the outer membrane. Work on bacteriocins has been driven by the need of new and better way of food preservation technology and a wish to prevent food spoilage and poison producing bacteria from growing. Also in recent years, a new perspective of bacteriocins i.e, probiotics has been emerged [24]. Infact, nisin and lactisin 3147 have been found to prevent mastitis [24]

VI. MODE OF ACTION

The bacteriocins which have gained importance in food preservatives are the lantibiotics produced by lactic acid bacteria. These groups of bacteriocins are positively charged molecules with hydrophobic patches. Chen et al in 1997 suggested that electrostatic interactions with negatively charged phosphate group on target cell membrane contribute to the initial binding of the bacteriocin with the target membrane [26]. The lantibiotics inhibit the target cells by forming pores by the interaction with lipid II molecule in the membrane causing the depletion of the transmembrane potential or pH gradient which results in the leakage of the cellular materials. This binding to lipid II molecule is involved in inhibition of peptidoglycan synthesis which in turn affects the cell wall formation and affects reproducibility. In case of class II bacteriocins such sakacin, the hydrophobic portion inserts into the membrane as transmembrane channel and forms pores. The association of hydrophobic patches of bacteriocins with the hydrophobic membrane has also been modeled using computer simulation by lins et al 1999 to predict the most favorable interaction [27]. There are two models by which the mode of action can be explained. The first one is the barrel stave and the other one is the wedge mechanism. In barrel stave, nisin orients perpendicularly to the membrane forming an ion channel that spans the membrane [28] and according to the wedge model, after a critical number of nisin molecules are attached to the membrane, they insert concurrently like a wedge. Also, the phospholipid composition and environmental pH influences the MIC [minimum inhibitory concentration] value [26]. Almost all bacteriocins have different modes of action. In some cases, it has also been seen that instead of pore formation, docking molecules on the target cell membrane facilitate the interaction with the bacteriocin, thereby increasing the effectiveness of the bacteriocin for eg. Mersacidin [5]. There are many factors which are responsible for bacteriocins to act accordingly for example, the environment in which the particular bacteria is residing.

VII. APPLICATION OF BACTERIOCINS

Bacteriocins firstly seen as analogous to antibiotics have been characterized more deeply and the potential applications of these compounds have also increased. Till date nisin is the only bacteriocin which has got the status of GRAS [generally regarded as safe] from FDA [food and drug administration] and is sold under the brand name of nisaplin^{tm} It can be used in packed food items as preservative in required amount as written in IP [Indian pharmacopeia] standards. Other applications where bacteriocins are gaining importance in the field of food are in packaging of food items such as chocolates, milk, curd etc. Bacteriocins may also be used as probiotics as a measure to improving the microbial flora of gut region. Different species of lactic acid bacteria eg leuconostoc, lactobacillus, pediococcus etc. have been used as probiotics. Within gut, the beneficial microflora helps in digestion and breaking down the toxic chemicals generated. It is noteworthy that in human gut, there exists both the beneficial and harmful microfloral population. It is said that to

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attain the healthy condition, the ratio of beneficial and the harmful concentration in the body should be 85:15. The intestinal microflora are the prominent members of probiotics and are found to help in reduced diarrhoeas, lowered blood cholesterol, increased immune response and prevention of cancer [28]. Bacteriocins can also be used in improving the livestock by providing the live culture as feed to cattles, fishes and poultry. New potential applications have also been found in medical field example mersacidin is found to have potential medical application in treating skin infections and acne. Yet another example is lacticin 3147 which inhibits streptococcus mutans and has the potential to inhibit tooth decay and gingivitis. Bacteriocins are also experimented to treat urinogenital and vaginal disorders as well.

VIII. NISIN

Nisin is a natural, toxicologically safe and antibacterial food preservative. It is found to show an antibacterial activity against a wide variety of gram positive bacteria and some spores. As far as the history is concerned, the term nisin was coined by Mattick and Hirsch in 1947. Hirsh and others in 1951 first examined the potential of nisin as the food preservative. In 1957, nisin was found to commonly occur in farmhouse cheese [12] and in 1969, nisin was approved for use as an antimicrobial in food by the Joint FAO/WHO Expert Committee on Food Additives [30]. Nisin has been given the food additive number 234 and is permitted for use in food preservatives in more than 50 countries. Since 1953, nisin has been sold under the name, nisaplin^{tm} which contains approximately 2.5% nisin and remaining are the milk products fermented by LAB. Nisin is also marketed under different brand names as GUARDIAN^{tm}, novacin^{tm}, novaGUARD^{tm}. Nisin works on the permeability mechanism in which it forms complex with the lipid molecule of the bacterial membrane and subsequently, pores are formed as the complex inserts in the membrane which results in the cell death. Nisaplin^{tm} is an extremely stable product which shows no or very less activity in dry conditions, dark and in 25^{o}C. It has an increased solubility in acidic environment but decreased solubility as the pH increases. The stability of nisin depends on three factors including the incubation temperature, length of storage and pH. Greater nisin retention occurs at low temperature. The food products where nisin can be used as food preservatives are dairy products which include cheese (processed), natural cheese, yogurt,; egg products and value added egg products including omelets', pancakes etc.; pasteurized soups, flour based products including crumpets; sea foods; canned foods; salad dressings; alcoholic beverages and meat products [30].

IX. BACTERIOCINS AS FOOD PRESERVATIVES

Many LAB bacteriocins are active against many food borne and spoilage gram positive bacteria including antibiotic resistant bacteria. The gram negative bacteria are intrinsically resistant due to protective role of external membrane; however some can be active in combination with other membrane destabilising agents like EDTA [29].Till date there are only some bacteriocins which are known to have been used as preservatives. Also the majority associated with food industry are the members of class I and class II. Among class I, nisin is the best known example which has gained GRAS status and can be used directly as the food ingredient having a broad inhibitory spectrum against a wide range of gram positive bacteria including pathogens. Also it is effective against clostridium and bacillus spores; however, it is not effective against the gram negative bacteria, yeasts and molds. Among the class II bacteriocins, pediocin having anti- listerial activity are most common. They are produced by pediococcus specie but not effective against the spores but can inhibit listeria monocytogenes effectively as compared to nisin. The pediocin is very much effective against the wide spectrum of gram positive bacteria (LAB), food borne pathogens and some spoilage bacteria including gram negative bacteria such as pseudomonas and E.coli [9].

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