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RESEARCH TITLE

BENEFITS AND MECHANISM OF ACTIONS OF BACTERIOCINS IN LACTIC ACID BACTERIA

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Abstract

Bacteriocins are the products of secondary metabolism of several bacterial genera that exhibit antimicrobial activity. Although there are many bacteria that produce bacteriocins today, no way has been developed how these biological molecules are classified. Many have not been authorized for therapeutic use against multi-antibiotic-resistant microorganisms due to their potential toxic effects, although recent studies have made progress in understanding and demonstrating their medical and biological importance and mechanisms of action against microorganisms, and so they can be optimally used to solve many health and food conservation problems. The objective of this review was to review information about bacteriocins, their types and mechanisms of action, and the applications of their use in the food, pharmaceutical and biotechnology industries.

Key Words: Bacteriocins, Inhibitory spectrum, Mechanism of action, Applications.

Introduction:

Lactic acid bacteria have recently attracted considerable attention of researchers for many benefits, as helping to maintain the natural and healthy balance of living organisms and practicing this balance by various means, such as producing of lactic acid and other acids, as well as the production of many small organic substances that have inhibitory effect of a number of microorganisms , which have been called bacteriocins [1]. The study of bacteriocins began in 1925 with investigation of a plasmid that carries a gene coding for the protein produced by *Escherichia coli*. This protein acts to kill other intestinal bacterial cells closely related to its producing bacteria [2]. In 1946, Gratia and fredricqe named Colicin for a protein produced by some of the *E. coli* strains. Colicin is considered as a complex compound of Lipopolysaccharide [3].

Bacteriocins were not only produced by members of the intestinal family, so these compounds , whether produced by gram negative or positive bacteria, were coined by the universal term bacteriocin . 90% of bacteria , including gram positive and negative bacteria , produce growth antibacterial substances which are peptides have antibacterial properties and are not categorically associated with antibiotics , as they differ from narrow-spectrum killing and are toxic to bacteria very close to the bacteriocin producing strain or species [4].

Identification of bacteriocins

Bacteriocins are antimicrobial bio-compounds of a protein nature , which may be combined with lipids or carbohydrates , produced by a large selection of bacteria , have an inhibitory effect , lethal or suppressive effect on the growth of sensitive bacteria that are genetically related to the bacteria produced them. The lethal effect is due to their connection with cell receptors that are sensitive to them. However, lactic acid bacteria are known as low molecular weight peptides that have antibacterial potentials and their range often extends to species other than those closely related to the producing bacteria [5] .

Several antibacterial proteins have been produced by a number of Gram-positive bacteria, using lactic acid bacteria as natural preservatives of food because they are considered to be healthy substances that are degraded by enzymes that break down proteins found in stomach secretions [6]. Bacteriocins are not considered harmful to humans or animals, as they are added to increase the shelf life of foods. Bacteriocins' mechanics are by piercing the cytoplasmic membrane, inhibiting the construction of the cell wall of the target cell, or inhibiting metabolic processes, such as stopping the construction of nucleic acids or proteins [3] .

Bacteriocin nomenclature

Bacteriocin nomenclature is based on derivation from the name of producing bacteria and addition of (cin) , such as pyocin is derived from *Pseudomonas pyocyaneae*, or may be based on inhibitory spectrum of producing bacteria. Seventeen species of Colicin have been differentiated based on tests of resistance to inhibitor, using a number of sensitive strains. Several years later the bacterial nomenclature was approved, depending on the species or genus of the bacteria producing it, the genes of the strain producing it, or the cells receiving the bacteriocin [3,5].

Inhibitory spectrum of bacteriocins

The inhibitory spectrum refers to the ability of bacteriocins to inhibit bacteria related to fully produced bacteria such as some species of the genus Lactobacillus that have inhibitory spectrum towards Gram-positive bacteria and less related bacteria such as *Staphylococcus aureus* and *Clostridium botulinum.* Nisin is a bacteriocin that has a wide inhibitory range

against a large number of Gram-positive bacteria and inhibits the growth of spores of Bacillus cereus and Clostridium botulinum and also inhibits pathogenic food-causing bacteria. The main foods in which lactic acid bacteria are found are yogurt and fruit flavored yogurt [7] .

Benefits of bacteriocins

Bacteriocins are considered one of the most important products of lactic acid bacteria. Lactic acid bacteria are one of the most famous probiotics used to maintain human health. It has long been used in the food, agricultural and other industries. As safe use in these areas has been demonstrated, these bacteria are also found as natural flora in some parts of the digestive tract. Lactic acid bacteria include several genera and species used as promoters and Lactobacillus is the most commonly used genus. The most important species of Lactobacillus are *LB. Acidophilus, LB. Gasseri, LB. Fermentum, LB. Bulgaricus* [8].

Biological promoters maintain and promote human health through their metabolic activity and release products through competition with pathogens, stimulation of the immune system, and minimization of side effects of antibiotic therapy. Bio-enhancers also have important roles in treating diarrhea, lowering blood cholesterol, constipation, etc., as well as in treating liver and urinary tract injuries [9,10].

Types of bacteriocins

Bacteriocins are classified according to production into two types: bacteriocins produced by Gram-negative bacteria, as bacteria produce many specifically named depending on genus or species, such as Colicin of species *E. coli*, while bacteriocins from the genus *Pseudomonas* are generally referred to as Pyocin. Most bacteriocins produced by Gram-negative bacteria are effective only against very close species [11]. The second type is bacteriocin produced from Gram-positive bacteria GR +, and this bacteriocin is produced by GR+ Lactic acid Bacteria (LAB) Such as Nisin, a protein antibiotic of *Lactococcus lactis*, which has been shown to be used as an additive in 50 countries , as Nisin is the leading bacteriocin for the variety of practical applications in the food industry and food conservation [12].

Chemical and physical properties of bacteriocins

The chemical nature of some types of bacteriocins was studied and it was found that they are simple protein compounds or conjugated with lipids and carbohydrates, but the active part which is attributed to the deadly effect of bacteria is of a protein nature and is broken down by proteolytic enzymes. Bacteriocins have a narrow, focused efficacy on nearby species, and lethal action due to the presence of special receptors on sensitive cells [13]. Bacteriocins are characterized by the presence of the genetic material responsible for their producing and resistant to it on the plasmid. The strains that do possess it are either resistant to or immune to the deadly effects of the bacteriocin, as well as, they are amphipathic, facilitating the process of interaction with sensitive cell membranes [14] .

Bacteriocins are positively charged ions that are not affected by negatively charged or non-ionic disinfectants and these biomaterials serve to permeate the cytoplasm membrane of cells that are sensitive to bacteriocin influence. Because bacteriocin is considered a protein substance, some bacteriocins of high molecular weights may use as excellent antigens. Also the low-molecular-weight bacteriocins are characterized by temperature and pH stability. Nisin, for example, is a bacteriocin that is highly effective over a wide range of pH and highly potent at high temperature [15].

Bacteriocin production genes

The genes of the plasmid or chromosome, or both , are responsible for the production of bacteriocins. The genes specialized in the production of bacteriocins are located in clusters on

synthetic gene and close to immune genes. Bacteriocin gene clusters consist of three strongly related genes most often found on the plasmid : Toxin gene, Immunity gene, and Lysis gene. The toxin gene is responsible for the production of bacteriocin and has potentials to kill target cells. The immune gene encodes a protein that protects the cell from the act of killing with a bacteriocin protein and the hemolytic protein produced by the lysis gene destroys the target cell [16].

Factors affecting the production of bacteriocins

Temperature is considered one of the most important factors affecting the production of bacteriocins in general. Optimal growth temperatures of bacteriocin-producing microorganisms increase the production of bacteriocin, and some researchers have shown that high temperatures completely stop production and sometimes the producing bacteria may lose production [3] .

It has also been found that the pH can affect the production process, and that some bacteria produce large quantities of bacteriocins based on their initial pH at growth and increase their production at pH near neutral. The ventilation of bacterial culture increases the amount of bacteriocin produced, especially in the case of staphylococcus bacteria, while most lactic acid bacteria grow in anaerobic conditions with presence of CO2[17].The determination of the incubation period sufficient to obtain the best amount of bacteriocin should be done experimentally, depending on the type of bacterium, method and trial conditions. Studies have indicated the loss of efficacy at long periods of incubation time. This phenomenon has been attributed either to the appearance of bacterial inhibitors or to the action of an enzyme or the reuptake of the bacteriocin by the producing cell [18] .

Bacteriocins are secondary metabolites. They are created during the stationary growth phase, but the best product in the culture can occur at some stages of growth, such as the logarithmic growth phase, and reaches above after 24 or 48 hours of incubation. Most bacteriocins are produced in complex environments, and the presence of carbon and nitrogen sources as well as vitamins and amino acids in the medium components can increase the production of bacteriocin [19]. Some proteolytic enzymes relatively affect the stability of bacteriocins such as trypsin and pepsin by analyzing peptide bonds and altering the amino acid chain, which causes them to lose their ability to perform bioactive [4].

Mechanism of action of bacteriocins

The mechanism of action of bacteriocins depends largely on the chemical structure of the bacteriocin and on the cells that receive these biochemicals. Bacteriocins differ in their mechanism of action on the target cell, one of these mechanisms is the effect of the bacteriocins on the cytoplasmic membrane, where the cytoplasmic membrane of Gram-positive bacteria is the primary target of action by creating openings or perforations in the cytoplasmic membrane that cause damage. Many bacteriocins demonstrate their effect on the cell wall by targeting intermediate substances involved in cell wall construction or by analyzing the internal Bridges of Pentaglycine that bind the murine in the cell wall (Fig. 1) [5].

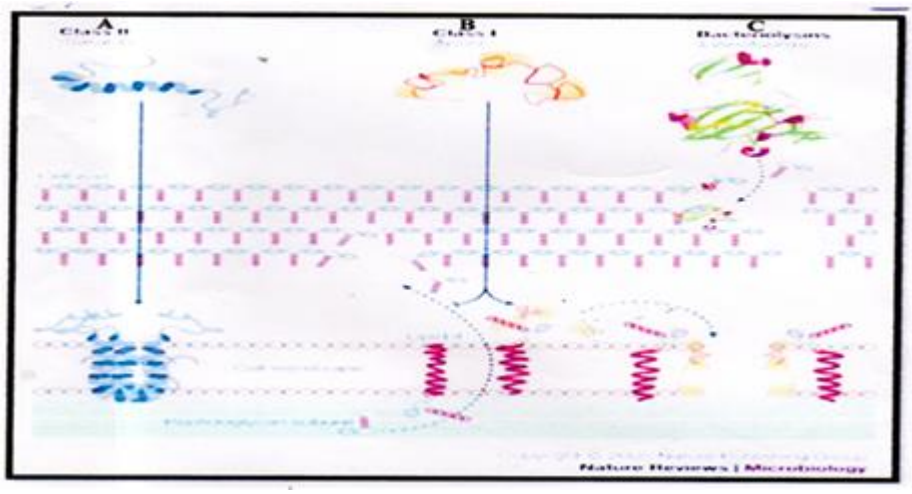

Figure 1. Illustrates how bacteriocin act in the wall and cytoplasmic membrane of a bacterial cell. **A.** effects on cytoplasmic membrane **B.** effects on cellular wall construction **C.** effects on inner wall bridge analysis[5].

Some bacteriocin species, such as Colicin, affect the biological synthesis of nucleic acids, and bacteriocin affect the bacterial species Bacillus cereus, which causes food poisoning, where it reducing the number of cells, inhibiting their ability to produce endotoxins, and increasing the sensitivity of bacteria to heat in heat-treated food samples [20].

Detection of bacteriocin production

There are many methods and techniques used to detect bacteria that produce bacteriocins, including immunological methods, genetic methods, and methods that depend on inhibitory effectiveness which are most commonly used, such as the Streak method. It is a quick and easy method , through inoculating the bacterial isolate that is to be studied for producing bacteriocin in a straight line in the middle of the dish, and after incubation and appearing of good growth , the bacteria to be tested are grown in lines perpendicular to the first inoculation line in the middle of the dish. After incubation, the production of the bacteriocin is detected by the observation of transparent areas around the productive bacterial colonies that represent areas of inhibition zones (Fig.2) [5,21].

Measuring of bacteriocin effectiveness

The most commonly methods used for measuring the effectiveness of bacteriocins are measuring the diameters of the inhibition zones by diffusion methods in agar plates, such as the well method, disk method, stain method, or a method of measuring the amount of growth inhibition in liquid cultures in terms of medium turbidity using a spectrophotometer after diluting the bacteriocin preparation into a series of graduated dilutions [3].

The inhibitory spectrum of bacteriocins is the group of microorganisms that bacteriocins can inhibit and most of which are characterized by a broad inhibitory spectrum, including bacteriocins of bacteria unrelated to gram-positive bacteria, pathogenic or food-damaging bacteria, and sometimes also including gram-negative bacteria. [22].

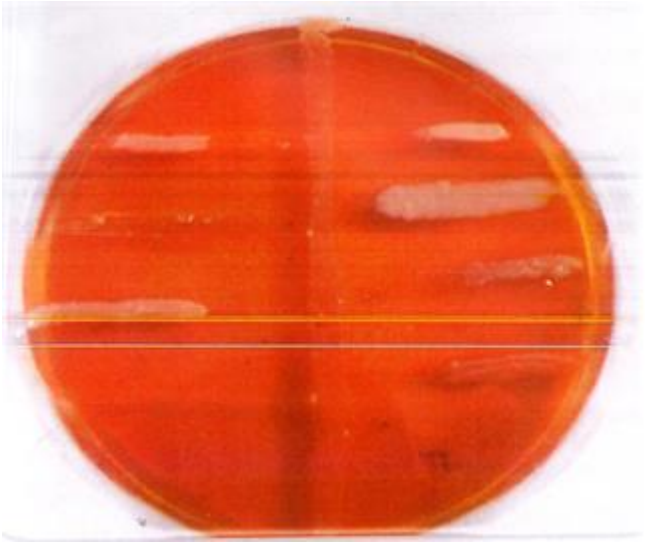

Figure 2. shows the transparent growth inhibition zone as evidence of bacteriocin production [5].

Purification of bacteriocins

The separation and purification of bacteriocins, means removing other substances and compounds present with the protein substance and obtaining it with a high degree of purity, to study their distinctive properties and improve their preservability when used in food, There are many methods used in purification that vary from bacteria to another , but there is basic steps , must be followed , include concentration of a bacteriocin supernatant at minimum volume through centrifugal and filtration of solid materials [15]. The second step is precipitation by several methods including the use of ammonium sulphate, polyethylene glycol (PEG) and organic solvents like alcohol or acetone , and the pH should be adjusted, as these biological products are sensitive compounds that are effective only under certain conditions of pH and temperature, care must therefore be taken in the selection and use of scientific methods for efficient separation of the product [23].

Food applications of bacteriocins

The production of a group of antibacterial proteins by many Gram-positive bacteria has attracted attention for their potential and use in food preservation and to reduce contamination of food with Gram-positive pathogenic bacteria, where the producing organism has some degree of self-protection against the antimicrobial material produced [24]. The use of commercial chemicals in food preservation causes fresh products to soften, while bio-conservation using the organism itself or its products lengthens the shelf life of the nutrients and supports the organic qualities of the food. Lactic acid bacteria have attracted attention that they are able to produce several healthy bacteriocins that are broken down by enzymes that break down proteins in stomach secretions, so that bacteriocins are not harmful materials to humans or animals [25]. Nisin is the most common bacteriocin that inhibits the growth of some bacteria that cause food spoilage and pathogenic bacteria that breed in foods such as *Clostridium, Bacillus*. Bacteriocins have been used as preservatives for cheese products - beverages - canned vegetables, and Nisin has been used for the treatment of peptic ulcers and for the control of mastitis in cattle. In addition , the bacteriocin was also prepared in powdered form and successfully added in children's diets [26,27].

Conclusion

The isolation and purification of bacteriocins for use as bio-preservatives in food is of great benefit as an alternative to unhealthy chemical antibiotics in the food industry. The Genetic improvement of producing bacteria increases their production of bacteriocins and can reduce the cost of production and access to obtaining bacteriocins with higher inhibitory effectiveness. The use of different additive technologies can increase the efficiency of bacteriocins in food preservation, such as different temperatures and times in pasteurization and sterilization, owing to the fact that bacteriocins can be produced on a commercial scale using cheap, readily available raw materials from factory waste, thus reducing production costs.

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