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# Innovative natural agents for inhibition of pathogenic bacteria and their resistant variants in vitro and in foods

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***ABSTRACT*** **: Antibiotics are produced by either bacteria or fungi. Actinomycetes produce two-thirds of the known antibiotics. Many of them are the third generations of certain classes of antibiotics, such as the β-lactam group. Unfortunately, many multidrug-resistant (MDR) microbes have recently been isolated from medicinal specimens or polluted foods. This showed that there is a need to find other innovative ways to inhibit MDR bacteria based on natural agents such as lactic acid bacteria (LAB), natural native and modified proteins, and nanoparticles singly or in combination with antibiotics. The resistance mechanisms to antibiotics are due to genetic determinants, the thickening of the bacterial cell wall, or modifications in specific site receptors on which antibiotics act. Also, certain reasons lead to resistance due to the ability of the bacterial pathogen to secrete enzymes that can degrade the antibiotic such as β-lactamases. Of the innovative natural agents which can inhibit bacterial pathogens are LAB; their metabolites such as organic acids, acetaldehydes, ethanol, diacetyl, and bacteriocins inhibit the bacterial pathogens and their resistant variants. Many genera of LAB, such as Lactobacillus, Lactococcus, Carnobacterium, Pediococcus, Leuconostoc, and Enterococcus, produce bacteriocins or antimicrobial proteins of broad spectrum activity against both Gram-positive and Gram-negative bacterial pathogen. Therefore, bacteriocins have been used recently for food preservation and have been shown to extend the shelf life of foods. Natural legume proteins, either native or modified by methylation, showed a broad spectrum antimicrobial activity, as the positive charges of methylated proteins attach negatively charged phospholipids of the bacterial cell membrane, making hydrophobic-hydrophobic interactions. This leads to the formation of pores in cell membranes, from which leakage of electrolytes occurs and causes cell death. In addition, nanoparticles have been recently used in combination with antibiotics in either medical therapy or in food preservation. In Egypt, the shortage of freshwater resources and their pollution constitutes a growing concern. Due to the uncensored use of pesticides in the agricultural regions of Egypt, the contamination risks of ground water increase periodically in planting seasons. Therefore, the present work aims to monitor the occurrence of organochlorine pesticides (OCPs) residues and heavy metals in five ground water samples collected from agricultural area with long-term pesticide application history in Belbis region, El-Sharqia, Egypt. Water samples were processed using a solid-phase extraction technique and gas chromatograph equipped with mass spectrometry (GC-MS). Results revealed that, the concentrations of OCPs in groundwater are in the limits except only 0.65 µg/L of p,p'-DDT recorded in ground water at Hassan Bieh village location, Belbis region, El-Sharqia, Egypt. Levels of iron and manganese in (Elnoba and Awlad Mahnaa) ground water samples were found to be much higher than the limits of Egyptian quality standards. The other elements in this study were found in the limits. The turbidity in 3 site (Hassan Beih, Elnoba and Awlad Mahnaa) villages has high values (4.7, 28.4 and 4.4), respectively. TDS values in two sites (Hassan Beih and Awlad Mahnaa) villages showing values above the 1000 mg/L limit.**

***KEYWORDS: Lactic acid bacteria (LAB); Antibiotics; Multi-drug resistant bacteria; Natural proteins; Nanomaterials*.**

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## I. INRODUCTION

The selection and characterization of MDR bacteria are recent challenges to find out other innovative, safe, natural agents to kill such MDR microbes (Ghada et al., 2020). Methicillin-resistant Staph. aureus (MRSA) are the ones known for many years (**Taisir et al., 2022**). These MRSA strains have been reported to be vancomycin resistant 10 µg/ml concentration (**Taisir et al., 2022**). Many vancomycin-intermediate Staph. aureus (VISA) were identified and inhibited by modified natural proteins **(Osman et al., 2016)**. Additionally, recent studies have shown that the Staph. aureus can resist the action of ≥15 µg/ml vancomycin, and such strains were isolated and identified as vancomycin-resistant Staph. aureus (VRSA) **( Taisir et al., 2022)**.

Recently, many other genera of bacterial pathogens showed multi-drug resistant of many antibiotics. Listeria monocytogenes is a dangerous pathogen where it can grow at different levels of pH values, at high salt concentrations (≥7%), and at low refrigeration temperatures **(Sawsan et al., 2018)**. These high potentialities of growth of such organisms make it rather dangerous. Many resistant variants of L. monocytogenes were isolated and characterized **(Enan, 2006a**). The resistances of L. monocytogenes were due to the modification of specific site receptors in the bacterial cell wall. These resistant strains of L. monocytogenes were inhibited by recent innovative natural agents such as bacteriocins, LAB, modified legume proteins, and nanomaterials (either singly or in combination with different antibiotics) **( Sawsan et al., 2018)**. Consequently, the modified natural proteins and bacteriocins have been used recently for food preservation to extend the shelf life of foods.

The resistance to antibiotics was shown to be either natural resistance (due to genetic reasons or thickening of the cell wall) or acquired resistance (since plasmid-mediated resistance genes can be transmitted from one organism to another) **( Wanda, 2018)**. The acquired resistances were detected in Streptococcus pyogenes, Enterococcus faecalis, Enterococcus faecium, and other bacterial species within the genus Bacillus. Many strains of E. coli were shown to be MDR bacteria, Klebsiella pneumonia, Pseudomonas aeruginosa, and Salmonella typhi **(Saha and Sarkar, 2021**). There is recently a current international challenge to inhibit the MDR bacteria. In this regard, bacteriocins, organic acids, modified legume proteins, and nanomaterials showed promising results in inhibiting these MDR bacteria and could be used for food preservation **(Abdel-Shafi et al.,2019, Enan et al., 2014).** LAB are Gram-positive and catalase-negative organisms that showed recent probiotic capability as they inhibited MDR bacteria by their metabolites such as lactic acid and other organic acids, diacetyl, ethanol, and bacteriocins **( Enan et al., 2018).** Hence, the LAB strains are recent and promising agents for inhibiting pathogenic bacteria at food processing. These bacteria can ferment foods such as Zabady, pickles, and sausage with the protection of the food products obtained with extended shelf life **(Reda et al., 2018)**.

Natural legume proteins such as glycinin and β-conglycinin are extracted and purified and showed promised antimicrobial activity against pathogenic bacteria in vitro and in food, including the MDR bacterial strains. Chemical modification of such proteins by either methylation or esterification and the addition of sulfur amino acids increased the antimicrobial activity of these proteins **(Abdel-Shafi et al., 2019)**. This is because the positively charged amino acid residues can attach to the negatively charged phospholipids' bacterial membranes, making pores that can discharge the cell contents outside cells, leading to cell death **(Sitohy et al., 2013)**.

Nanomaterials are particles in the 1-100 nm scale range and can be synthesized by physical, chemical, and biological methods **(Gurkok and Ozdal, 2021)**. These nanoparticles showed promising antimicrobial activity and inhibited MDR bacteria. The nanoparticles interfere with the bacterial cell membrane, increasing permeability, and cell death. Recently, studies have been conducted to use nanoparticles in combination with antibiotics, naturally modified proteins, or LAB metabolites, as they could work together in synergism reflecting higher antimicrobial activity **(Sitohy et al., 2021)**. The present review discusses the recent challenges caused by the appearance of MDR pathogenic bacteria. The innovative natural agents used to control the growth of MDR bacteria using LAB metabolites, natural legume proteins and nanoparticles are discussed.

## II. SELECTION OF MULTIDRUG-RESISTANT (MDR) MICROBES IS A CHALLENGE TO FIND OUT INNOVATIVE NATURAL AGENTS FOR INHIBITION OF THESE MDR MICROBES

### 2.1. Antibiotics and the recent challenge faced their action:

Antibiotics are produced by microorganisms such as non-filamentous and filamentous bacteria and fungi that kill other indicator bacteria (antibacterial antibiotics) or fungi (antifungal antibiotics). Unfortunately, many resistant variants of microbes were isolated and characterized and resisted the action of antibiotics **(Davies, 2007)**. This is a

recent challenge to continue research to find other natural agents that could kill the MDR microbes. These agents are LAB, natural proteins, bacteriocins, and nanomaterials (**Enan and Amri, 2006)**.

There are several methods of action through which antibiotics exert their impacts. Most are beta-lactam antibiotics, and they function by stopping the production of cell walls in bacteria. Synthesis of the bacterial cell wall entails partial construction of wall constituents inside the cell, the passage of these components across the cell membrane to the developing wall, the creation of the wall, and cross-linking of wall strands. Antibiotics that limit cell wall production have a particular impact on one or more phases. The outcome is a change in the cell wall and morphology of the bacterium and, ultimately, the bacterium's mortality **(Nikaido, 2009).**

### 2.2. Susceptibility and resistance of pathogenic microbes to antibiotics:

It evaluates the drug's capacity to eradicate microorganisms. The findings from this examination may allow physicians to predict which medications are anticipated to be more helpful in curing disease. Typically, sensitivity evaluation is conducted in a clinical lab using culture techniques that subject microorganisms to antibiotics or genetic procedures that determine if the microorganisms possess genes conferring resistance. Determining the width of regions lacking growth of bacteria, known as inhibition zones surrounding paper discs carrying antibiotics on agar culture plates equally loaded with bacteria, is a common step in culture procedures. The diameter of the inhibition zone may be used to determine the minimum inhibitory concentration (MIC), the minimum concentration of an antibiotic that inhibits bacterial growth (**Nijs *et al.*, 2000**).

Since the beta-lactam antibiotic penicillin emerged, an antimicrobial susceptibility assessment test has been necessary. Initial procedures utilized dilution or culture and were phenotypic. Since the 1980s, the E- test, which consists of an antibiotic-impregnated strip, has been accessible, while genetic approaches such as polymerase chain reaction (PCR) analysis have been widely applicable since the early 2000s (**Bolmström *et al*., 1988**).

### 2.3. Mechanism of resistant bacteria to antibiotics:

Cloete **(2003)** defined resistance as the persistent or temporary capacity of an organism and its offspring to stay alive or reproduce under circumstances that would kill or hinder other organisms of the strain **(Cloete, 2003).** Microorganisms are developing a high level of resistance to the majority of known antibiotics, notably, Gram-negative rods (e.g., *Salmonella spp.*, *Escherichia coli*, *Klebsiella oxytoca*, *Acinetobacter spp.*, and *Pseudomonas aeruginosa*), which are resistant to nearly all existing antibiotic treatment. Resistance and virulence may function as a potentially lethal duet, as demonstrated in the recent massive epidemic outbreak of *E. coli* O104:H4 in Europe, particularly Germany (**Buchholz *et al.*, 2011**), where the antibiotic supply has become critically depleted (**Hughes, 2011**). Therefore, antibiotic therapy for *E. coli* cases is prohibited **(Abdel-Shafi *et al.*, 2016**). Bacteria resist the antibiotic action via one or more of the following mechanisms:

#### 2.3.1. Pump the antibiotic out from the bacterial cell:

Bacteria can produce pumps that sit in their cell wall or membrane. These so-called efflux pumps are widespread in bacteria and can transfer many substances, including signal molecules and micronutrients. Several of these pumps may expel antibiotics from the cell membrane, decreasing the antibiotic level within the bacterial cell. In certain instances, changes in the bacterial DNA might cause the bacterium to generate more of a particular pump, hence increasing resistance (**Cox and Wright, 2013; Sun *et al.*, 2014**).

#### 2.3.2. Reduce the permeability of the bacterial cell's surrounding membrane:

Specific modifications to the bacterial cell membrane render it harder to penetrate across. Thus, fewer antibiotics are delivered to the bacterium **(Cox and Wright, 2013).**

**2.3.3. Destruction of the antibiotic:**

Antibiotics may be rendered ineffective by bacterial enzymes. The enzyme β-lactamase, for instance, degrades the bioactive constituent (the β-lactam ring) of penicillin, one of the most vital pharmaceuticals for curing infections in humans. Bacteria that create extended-spectrum β-lactamases, often known as ESBL- forming bacteria, have emerged as a significant issue in recent years. They may destroy a broad range of β-lactam drugs, including even the last-resort treatments for illnesses caused by these bacteria **(Bolmström *et al.*, 1988).**

**2.3.4. Modification of the antibiotic:**

Occasionally, bacteria can produce enzymes that add new chemical groups to antibiotics. This prevents the antibiotic from attaching to its receptor in the bacterium **(Morris *et al.*, 1984).**

**2.3.5. Camouflaging of the target:**

Due to DNA mutations, modifications in the content or architecture of the target in the microorganism might prevent the antibiotic from engaging with the target. Alternatively, the bacteria may add various molecular groups to the structure of the target, protecting it against the antibiotic **(Morris *et al.*, 1984).**

**2.3.6. Expressing various protein alternatives:**

Several bacteria can produce alternate proteins that might be substituted for those blocked by the antibiotic. *Staphylococcus aureus*, for instance, may adopt the resistance gene *mecA* and generate an alternative penicillin-binding protein. β-lactam antibiotics attack these proteins, which are required for bacterial cell wall production. The altered penicillin-binding protein's poor affinity for β-lactam antibiotics renders the resistance of bacteria to the medications, allowing them to tolerate the medication. MRSA (methicillin-resistant *Staphylococcus aureus*) is based on this form of resistance **(Abdel-Shafi *et al.*, 2013).**

**2.3.7. Target reprogramming:**

Frequently, bacteria may generate a variation of a necessary structure. In contrast to antibiotic-susceptible bacteria, those resistant to vancomycin produce a cell wall that is much thicker and resistant to the drug. This form of the cell wall is less susceptible to antibiotic interaction **(Taisir *et al.*, 2022).**

Certain bacteria have an inherent resistance to specific antibiotics. Consider, for instance, an antibiotic that damages the bacterial cell wall. The antibiotic will be ineffective if a bacteria lack a cell wall. The term for this phenomenon is intrinsic resistance. It is referred to as acquired resistance when a bacterium formerly sensitive to an antibiotic develops resistance. Bactericidal and bacteriostatic drugs are the two basic groups that distinguish the mechanisms of antibiotics. A bactericidal antibiotic disrupts vital functions, destroying the cell. The peptidoglycan building blocks required for cell wall formation are the targets of a wide variety of antibiotics (acting as bactericides) authorized by the Food and Drug Administration **(Oka *et al.*, 1980)**.

Over fifty percent of the antibiotics employed in clinical circumstances are β -lactams. By covalently altering a crucial serine residue on the active side of cell-anchored transpeptidases, β-lactam antibiotics efficiently inhibit cell wall cross-linking, reducing peptidoglycan integrity and causing cell damage. Vancomycin and other glycopeptides inhibit peptidoglycan formation by binding and sequestering lipid II precursor components. Lipid II components are essential for transporting biosynthesized monomeric peptidoglycan building blocks from the cytosol to the mature peptidoglycan scaffold **(Cloete, 2003).**

Cell wall production is halted by preventing the transfer of monomeric peptidoglycan building blocks from the cytosol to the developing scaffold on the bacterial cell surface. Antibiotics that attack repair enzymes and bacterial DNA replication are also classified as bactericidal. These medicines block enzymes, such as DNA gyrase and topoisomerases, that are necessary for the survival and proliferation of bacterial cells. In this family of antibiotics,

fluoroquinolones are regarded as broad-spectrum medicines that are potent for both G-positive and G-negative bacteria **(Sabulski, 2017)**.

In contrast to bactericidal antibiotics, bacteriostatic medicines inhibit bacterial proliferation without causing cell damage. These antibiotics work against bacteria by exploiting the structural features of ribosomal subunits and other components required to initiate, elongate, and terminate protein products unique to bacteria and not found in eukaryotes. Antibiotics like tetracyclines and aminoglycosides, which the Food and Drug Administration has authorized, belong to this family. They work by inhibiting the ribosome's translational machinery, stopping the production of proteins. Clindamycin, for instance, is often recommended for treating *S. aureus* infections **(Sabulski, 2017).**

## III. BIOLOGICAL AND TECHNOLOGICAL PROPERTIES OF LAB

LAB are Gram-positive and catalase negative. They can use the carbohydrates as the only or main carbon source (**George *et al*., 2018)**. Their metabolites are lactic acid and other organic acids. LAB are commonly found in foods, including fermented vegetables, fruits, beverages, and dairy products. Currently, LAB includes the Genera *Lactobacillus, Leuconostoc, Pediococcus, Carnobacterium, Lactococcus, Enterococcus, Aerococcus, Vagococcus,* and *Streptococcus* **(Nettles and Barefoot, 1993; Reda *et al.*, 2018).**

### 3.1. Types of LAB:

***A. Lactobacilli*:**

Lactobacilli include three groups. Group 1 includes the obligatory homo-fermentative lactobacilli, which can degrade hexose but cannot pentose. Group 2, the facultative hetero-fermentative lactobacilli, triggers the fermentation of hexoses almost exclusively to lactic acid, acetic acid, and aldehyde or ethanol. Group 3, the obligatory hetero-fermentative lactobacilli, ferments hexoses and pentoses to lactic acid and ethanol **(Enan *et al*., 1996)**. *Lactobacillus plantarum* is a versatile and industrially important lactic acid bacterium found in fermented pickles. It showed probiotic capabilities (**Hebert *et al.*, 2000)**. Its probiotic properties include resistance to biological barriers, antimicrobial activity, and cholesterol-lowering effect (**Hebert *et al.*, 2000**). The antimicrobial activity enables this bacterium to protect the produced pickles. This antimicrobial activity of *L. plantarum* is due to its secretion of lactic acid and bacteriocin **( Enan, 2006; Ismaiel *et al*., 2013)**

***B. Leuconostoc:***

The cells of the Genus *Leuconostoc* are spherical or lenticular and occur in pairs or chains. They are facultative anaerobes. *Leuconostocs* are morphologically different from lactobacilli **(Garvie, 1986)**. However, physiologically they are quite similar to the gas-producing heterofermentative lactobacilli (**Holzapfel and Schillinger, 1992)**. The classification and identification of the *Leuconostocs* have therefore been equivocal. The studies of DNA: DNA homology and rRNA similarity (**Garvie, 1981)** differentiated the *Leuconostoc* species from lactobacilli. These studies added two new genera: *Leuconostoc paramesenteroides* and *Leuconostoc oenes*, to the genus *Leuconostoc* **(Martinez-Murcia and Collins, 1991).**

The Genus *Leuconstoc* includes the following species: *Leuconstoc mesenteroides, Leuconstoc dextranicum* **(Zheng *et al*., 2020),** *Leuconstoc cremories* (**Knudsen and Sorensen, 1929**), *Leuconstoc lactis* (**Garvie, 1967)**, *Leuconstoc mesenteroides* subsp. *amelibiosum* (**Hoover, 2000)**, *Leuconstoc gelidum* and *Leuconstoc carnosum* (**Shaw and Harding, 1989)**, *Leuconstoc fallax* **(Martinez-Murcia and Collins, 1991),** and *Leuconstoc amelibiosum* (**Schillinger *et al.*, 2001)** which was formerly *Leuconstoc mesenteroides* subspecies a*melibiosum*. *Leuconstoc dextranicum* and *Leuconstoc cremoris* are now considered subspecies of *Leuconostoc mesenteroide*s (**Pot *et al*., 1994)**.

In food technology, *Leuconostoc spp.* are often used in conjunction with rapid acid-generating *Lactococcus spp.* as undefined mixed kind starting cultures, leading to fragrance and texture development of the end food items via citrate breakdown and synthesis of acetoin, diacetyl, and $CO_2$ (**Özcan *et al.*, 2019)**. *Leuconostoc mesenteroides* is referred to as "the aroma bacterium" because of its ability to produce aromas (**Starrenburg and Hugenholtz, 1991**). *Leuconostoc spp.* are distinguished by heterolactic fermentation via the phosphoketolase route, which produces fragrance and taste components, including lactic acid, ethanol, acetic acid, and carbon dioxide **(Özcan *et al*., 2019).**

### *C. Carnobacterium:*

To provide accommodation for non-aciduric *Lactobacillus piscicola* and *Lactobacillus divergens* species, **Collins *et al.* (1987**) suggested the Genus *Carnobacterium.* The latter two species (atypical lactobacilli) were differentiated from *Lactobacillus spp.* by their lack of growth on acetate agar, the growth at high pH values of 8.5-9.5, and the synthesis of oleic acid instead of cis-vaccenic acid obtained by lactobacilli (**Collins *et al.*, 1987)**. Based on these biological properties, these two atypical lactobacilli were classified as *Carnobacterium piscicola* (previously named *Lactobacillus piscicola*) and *Carnobacterium divergens* (originally known as *Lactobacillus divergens*). **(Collins et al. 1987**) identified two distinct species, *Carnobacterium vmobile* and *Carnobacterium gallinarum* **(Collins *et al.*, 1987).** The four species mentioned above of *Carnobacteria* had a considerable level of rRNA sequence similarities and constituted a phylogenetically cohesive cluster, distinct from all LAB (**Wallbanks *et al.*, 1990)**.

From the recommendations reported by (**Collins *et al.*, 1990)**, both *Lactobacillus maltaromicus* and *Carnobacterium maltaromicus* had 100% rRNA structural similarities, and the *maltaromicus* species was identified before *Lactobacillus piscicola* **(Hiu *et al.*, 1984).** Therefore, these two should replace *Lactobacillus piscicola* and *Carnobacterium piscicola* **(Miller *et al.*, 1974).**

### *D. Enterococcus:*

*Enterococcus* comprises the enterococcal group of streptococci (formerly faecal streptococci), possessing the group D antigen **(Schleifer and Kilpper-Balz, 1984).** Many species of the genus *Streptococcus* have been classified as enterococcal organisms belonging to the enterococcal group **(Jones *et al.*, 1972)**. The recent identification of two species, *Enterococcus faecium* and *Enterococcus faecalis*, of the Genus *Enterococcus* was approved **(Schleifer and Kilpper-Balz, 1984).** Since the name change in 1984, seventeen more species have been transferred to or validly described as *Enterococcus* **(Pot *et al.*, 1994).**

### *E.* Other LAB Genera (*Pediococcus, Aerococcus, Tetragenococcus, and Alloiococcus*):

Cells of these Genera are morphologically similar. They are spherical, Gram-positive, separate into tetrads by dividing the plane in half at right angles, non-motile, and facultative anaerobic, and require a rich medium containing complex growth factors to grow. All these genera species grow at 30°C, but optimum temperatures range from 25-40°C **(Pot *et al.*, 1994**). At present, the genus *Pediococcus* includes eight species. However, *Aerococcus* comprises only one species, *Aerococcus viridans* (**Pot *et al.*, 1994**).

Simple physiological or morphological examinations cannot distinguish between the individuals of both genera. DNA:DNA hybridizations (**Dellaglio *et al.*, 1981**) revealed associations between phenotypically identical bacteria such as *Pediococcus urinaequi* and *Aerococcus viridans*. *Pediococcus halophilus*'s classification as a member of the genus *Pediococcus* has been contentious due to its morphological similarities with *Aerococcus viridans* rather than the *Pediococci*. However, no intrageneric associations could be determined using DNA:DNA hybridizations **(Dellaglio *et al.*, 1981).** By comparing the 16S rRNA sequences of all *Pediococcus* species (excluding *Pediococcus inopinatus*) with those of *Aerococcus*,

**Collins et al. 1990** resolved this taxonomic issue, and *Pediococcus halophilus* was subsequently reassigned to the distinct Genus *Tetragenococcus* as *Tetragenococcus halophilus* **(Collins *et al.*, 1990; Pot *et al.*, 1994).**

One of the more recently recognized LAB Genera is *Alloiococcus* **(Aguirre & Collins, 1992)**, which was named after a previously unidentified bacterium identified from the middle ear secretions of children with chronic otitis. Just one species was reported for this genus.

### 3.2. Importance of LAB in the food industry:

LAB are technologically important organisms as they are used as starter cultures for food fermentation (**Nettles and Barefoot, 1993**). Many species of LAB such as Lactococcus lactis spp.lactis are involved in yoghurt making; Lactobacillus bulgaricus is involved in making of soft cottage cheese and other Karish cheese **(Ola *et al.*, 2021).**

Fermented fish was traditionally produced and is recently made using *Carnobacterium piscicola* and *Pediococcus acidilactici;* fermented fruits and beverages are produced industrially and currently by many species of *Lactobacillus brevis, Lactobacillus acidophilus, Lactobacillus curvatus*, and *Pediococcus pentosaceus* (**Kusmarwati *et al*., 2020)**. The attractive perspectives are the use of LAB as starter cultures to ferment foods and to protect such food produced by their ability to produce inhibitory agents **(Enan *et al*., 2018).**

For long-term storage, food may be pickled using lactic acid bacteria (LAB) anaerobic fermentation in brine or vinegar. The resultant product is known as a pickle. This process imparts the food with a salty or bitter flavor **(Islami *et al*., 2009)**.

Fermented pickles come in a wide range of flavors and textures. Their production can be broken down into several distinct categories: alcoholic fermentation (rice and cassava), lactic fermentation (fruits, vegetables, milk, meat, and cassava), mold fermentation (soybeans and peanut press cake), and high salt fermentation tauco (fermented soybean slurry), soy sauce, and fish (**Nuraida, 2015**). Pickles have been fermented since ancient times, and although traditionally they were handcrafted foods generated by spontaneous fermentation, modern methods have evolved to solve quality, safety, and mass production challenges. This requires the management of microbiological habitats, raw materials, and fermentation procedures (**Lan *et al*., 2013**). For fermented pickles, for example, *Lactobacillus* strains have been suggested as a handy starting culture.

Certain lactobacilli species are hetero-fermentative and capable of transforming hexoses (such as lactose and glucose) into lactic acid and then acetic acid (**Zaunmüller *et al*., 2006)** while creating other metabolites with beneficial characteristics. In addition, LAB-fermented pickles have a unique taste and beneficial health benefits ( **Kandasamy *et al*., 2018**). Several fermented pickles were identified as excellent sources of vitamins, proteins, minerals, carbs, and dietary fibers **(El Sheikha and Hu, 2020).** LABs might create bacteriocins, fragrance components, and exopolysaccharides (EPS) that contribute to flavor, texture, and prolonged shelf life in fermented pickles ( **Suzuki *et al*., 2013)**.

Moreover, LABs in pickled foodstuffs play a crucial function in detoxifying virulent/toxic production and degradation of mycotoxins, decreasing several health hazards. LABs have GRAS (generally recognized as safe) designation and are beneficial for combating the growth of infections and spoiling germs in pickled foods (**El Sheikha and Hu, 2020;)**.

### 3.3: Biocontrol of food-borne pathogens by LAB:

Lactic acid is the main metabolite produced by LAB to inhibit bacterial pathogens. LAB produce also other organic acids such as propionic, a decrease in pH, the accumulation of hydrogen peroxide and some strains were able to produce antimicrobial compounds **(Caplice and Fitzgerald, 1999).** In addition, the antifungal activity of LAB has other qualities that make them attractive candidates for the biological control of infections. Already accessible from the food business is the technology for the mass manufacture of LAB. In addition, LABs are deemed harmless for animal and human well-being since they are currently used in the food industry.

Apart from organic acids, LABs can produce and expel inhibitory compounds **(Daeschel, 1989)**. These compounds are hostile to a broad range of bacteria and may thus contribute significantly to the preservation activity of chemicals like diacetyl reuterin. Formic acid, ammonia, free fatty acids, hydrogen peroxide, ethanol, diacetyl, acetone, acetaldehyde, 2,3-butanediol, bacteriolytic enzymes, benzoate, classical antibiotics, bacteriocins, and a few other less well-defined or entirely unspecified inhibitory compounds are all generated, albeit in many tiny portions, than acetic or lactic acids **( Vuyst and Vandamme, 1994).**

Several of these chemicals are hostile to food spoilage organisms and food-borne microorganisms such as *Clostridium sporogenes, Bacillus cereus*, *Clostridium botulinum*, *Clostridium perfringens*, *Staphylococcus aureus*, and *Listeria monocytogenes* (**Vuyst & Vandamme, 1994) .**These antagonistic substances of LAB are of great interest in improving starter cultures for food fermentations (**Villani *et al*., 1993)**. They could be exploited to extend the shelf life of many foods (**Jiménez-Díaz *et al*., 1993)**. In addition, the LAB producers are used as probiotics since they neutralize bacterial pathogens, lower the blood cholesterol level, produce protease, lactase, and other enzymes, and can attach the smooth epithelial tissues **(Abdel-Shafi, *et al*., 2013).**

### 3.4. Bacteriocins of LAB and their technological properties:

The word bacteriocin was created by **(Jacob and Wollman, 1953)** to designate the highly specific antibacterial proteins produced by bacteria. One of the first identified bacteriocins was the colicins produced by *Escherichia coli*. Consequently, the shared properties of colicins significantly impacted the initial definition of the name "bacteriocin." Thus, bacteriocins were first classified as bacteriocidal proteins with fatal production, a restricted range of activities, and adhesion to particular receptors on cell surfaces (**Reeves, 1965**).

However, the emerging research indicated wide-ranging bacteriocins( **Goyal *et al.*, 2018)**. A further modification included the connection of bacteriocin production with plasmids (**Franklin and Snow, 1975**). In describing bacteriocins, the characteristics mentioned above have been used in varying configurations and implemented with varying levels of consistency and confirmation. They may have been applied to colicins in general. There were, nevertheless, variations in the bacteriocins generated by various Gram-positive bacteria. These entailed a broader range of action towards organisms of various types and a weaker host cell immune response to the same bacteriocin **(Nettles and Barefoot, 1993)**.

The proteinaceous nature and the bactericidal mode of action were typically relevant to well-characterized Gram-positive LAB bacteriocins. Only the inducible bacteriocins were discovered to be produced by lethal biosynthesis. Therefore, there were two issues with the phrase bacteriocins: (i) this category of compounds did not have a broadly agreed definition; and (ii) the majority of the documented inhibitory compounds have not been characterized to meet any categorization **(Eckner, 1992).**

Consequently, the word bacteriocin was assigned provisionally to those bacterial inhibitory compounds that have been demonstrated to satisfy at least two requirements: (i) the existence of an important physiologically active protein component; and (ii) a mechanism of bactericidal activity **(Tagg *et al.*, 1976)**. Those above two minimum criteria proved to be a precise definition for bacteriocins and are still used for the designation of antibacterial substances as typical bacteriocins ( **Nettles and Barefoot, 1993)**. A few antimicrobial proteins showed a bacteriostatic mode of action or non-proteinaceous nature and were, therefore, identified as atypical bacteriocins (**Grinstead and Barefoot, 1992)**.

Bacteriocins of LAB are currently used as food preservatives for meat and dairy products after FDA approved of the bacteriocins nisin as food safe preservative; it referred to nisin as GRAS status or Generally regarded as safe **(Nettles and Barefoot, 1993).** Since this date many bacteriocins were characterized and are used as safe food preservatives such as pediocin ACH,lacticin,plantaricin UG1 (**Enan *et al*.,2018).**

The bactericidal mechanism of functioning of bacteriocin is a second basic criterion for the characterization of a bacteriocin after the proteinaceous nature ( **Nettles and Barefoot, 1993**). Some bacteriocins are bacteriostatic and therefore considered atypical (**Grinstead and Barefoot, 1992)**.

A bacteriocin's action relies on the indicator bacteria's physiological status (**Vaughan *et al.*, 1992**). Most LAB bacteriocins are active only on log-phase cells of the test bacterium. These bacteriocins are so-called ion-channel forming bacteriocins, which are less active on stationary-phase cells than log-phase cells because they need a transmembrane potential to function **(Jiménez-Díaz *et al.*, 1993)**. However, few bacteriocins produced by LAB are active on both stationary- and log-phase cells. It is possible to assume that these energy-depleted cells (stationary-phase cells) are not completely de-energized so that the bacteriocin may commence its effect **(Zajdel *et al.*, 1985)**. In general, the activity of bacteriocin is more pronounced if susceptible bacterial cells are immersed in a buffer instead of an adequate broth, reflecting the protective function of the broth **( Enan, 1995)**.

Following a commonly established theory of the method of function of bacteriocins, interactions between bacteriocin and a susceptible cell occur in two phases. The first step involves the attachment of bacteriocin to the surface of the cell of a susceptible bacterium. The elimination of bacteriocin seems to leave the cell physically unharmed since no persistent physicochemical damage is generated. After a known time, the 2$^{nd}$ phase progresses, characterized by lethal biochemical changes. In this stage cytoplasmic membrane initiate reactions which cause a disruption of its electrochemical gradients by pore formation, causing its proton motive force to dissipate and so impeding energy generation and biosynthetic pathway of nucleic acids or proteins **(Brink *et al.*, 1994)**.

With nisin, a rapid, non-specific efflux of amino acids and cations and loss of membrane potential have been observed (**Eckner, 1992**). These biochemical reactions result in cell death (**Morries *et al.*, 1984**). Nisin does not need a membrane receptor to be activated but rather a functional membrane, and this function appears to be phospholipid-dependent (**Sahl, 1991)**. FDA has granted nisin, a bacteriocin generated by several strains of *Lactococcus lactis*, GRAS designation, meaning that it may be used without worry in food processing **(Negash and Tsehai, 2020). I**n contrast, several bacteriocins may improve plasma membrane permeability in a protein-mediated, voltage-independent mechanism. Some bacteriocins trigger the disintegration of the proton motive force and its subcomponents (**Klaenhammer *et al.*, 1993)**.

All the typical LAB bacteriocins show a bactericidal action method that may or may not include cell lysis. The above discussions are applied to the mode of action of the bacteriolytic bacteriocins **(Stoffels *et al.*, 1992)**. In contrast, the bactericidal action of non-bacteriolytic bacteriocins suggests that their modes of action are impairment of cell wall biosynthesis **(Brink *et al.*, 1994)**.

## IV. CONTROL OF PATHOGENIC BACTERIA BY NATURAL AGENTS

Natural agents are compounds with a wide structural diversity and remain an important source of new chemical compounds. Regardless of the growing emphasis on biological macromolecules, chemistry, and varied chemically synthesized laboratory technologies by pharmaceutical businesses and funding organizations, natural agents remain a significant supply of novel chemicals, novel therapeutic prototypes, and new pharmaceuticals **(Abdel-Shafi *et al.*, 2020).**

Natural agents are a vital supply of novel chemical variety and an indispensable part of the pharmacological encyclopedia of the present day. To overcome these difficulties, natural agents update future antibacterial medication prospects. Drug manufacturers have lately amplified their efforts to create novel antibiotics, which has become an urgent concern (**Amábile-Cuevas, 2003**). Nevertheless, several antifungal and antibacterial medicines now available have undesired cytotoxicity. The extensive use of these treatments has resulted in the fast creation of drug-resistant bacteria, which are the major reason for failure in medical and agronomic purposes. Thousands of microbial compounds have been identified, with many holding promises for therapeutic use (**Enan *et al.*, 1996**)

Legumes were used mostly as entire seeds, but in the past few years, the use of legumes in various forms (such as concentrate, flour, and isolate) has gained popularity (**Doxastakis, 2000**). Glycinin (also known as 11S globulin) and b-conglycinin (also known as 7S globulin) account for 34% and 27%, respectively, of the total protein in soy protein isolate, a typical instance of legume proteins **(Iwabuchi and Yamauchi, 1987)**. Soybean and other legume protein constituents are functionally and practically equivalent, according to research published by **Barker *et al.* (1976).** Antimicrobial proteins mediate pore-forming functions predominantly in membranes and are contributors to the development of innate immunity due to their distinct structural features **(Boman, 2000)**.

Since their identification more than three decades ago **(Boman *et al.*, 1974)**, peptides with antimicrobial properties have been categorized into various subgroups (**Boman, 2003**), such as alpha-helical peptides, peptides containing cysteine bonds, and peptides nourished in one or more amino acid residues. The capacity of antimicrobial peptides to infiltrate and alter target membranes is a distinguishing characteristic (**Shai, 2002**). Furthermore, it is thought that antimicrobial peptides destroy germs by non-receptor-mediated methods.

However, other peptides, like nisin Z, attach to the bacterial cell wall components and kill the germs in this way. Amphipathic structures are acquired by random-structured monomeric peptides when they form oligomers in solution, with the hydrophobic portions being encased in the oligomer's lumen and the hydrophilic parts being accessible to the solution, as stated by **Shai (2002)**. When a structure reaches a membrane, its organization is inverted. The membrane's lipid composition is in direct contact with the hydrophobic sections. If the peptides are laid out on the membrane surface and inserted using the 'carpet' process, the hydrophilic areas will be accessible to the solution (**Pouny and Shai, 1992**).

In the case of peptide oligomerization and membrane insertion through the "barrel" process, the hydrophilic areas are compartmentalized inside the oligomer's lumen (**Ehrenstein and Lecar, 1977)**. Regarding related antimicrobial peptides, two distinct peptide insertion methods have been suggested **( Chen *et al.*, 2003**).

**4.1. Modification of native proteins:**

Antimicrobial proteins and peptides (AMPPs) are among the recommended leading chemicals to combat microbial resistance. Even though antimicrobial peptides have not yet reached the antibacterial strength of modern medications, they are getting closer. They possess benefits that render them more intriguing. These benefits include **(a)** the capacity to prevent the formation of a bacterial resistance strategy by using a method of action that is not particular, **(b)** a diverse spectrum of activities, and **(c)** possessing a low level of host toxicity. In the last several years, more medications have been created to complement AMPPs.

AMPPs often include many cationic amino acids (**Sakamoto and Hamachi, 2018**). A widespread belief is that they work by nonspecifically attaching to cellular membranes. However, the precise mechanism of these interactions remains unknown (**Shadish and DeForest, 2020**). Modern biotechnology permits the production of cationic antibacterial proteins and peptides (CAMPPs). CAMPPs may be manufactured using one of four processes: enzymatic hydrolysis, chemical treatment, solvent extraction, and microbial fermentation of dietary proteins (**Alasalvar *et al.*, 2011)**.

Among the first strategies used to examine structure-function correlations was the chemical alteration of endogenous proteins. Esterification is a crucial and straightforward method for modifying proteins. Esterification inhibits free carboxyl groups, increasing the positive charge and turning the transformed protein more acidic (**Sitohy *et al.*, 2001**). Overall, the antimicrobial and antibacterial properties of protein and peptide molecules are improved by making them more positively charged.

Bovine lactoferrin was shown to be more efficient against a wide variety of Gram-negative and Gram-positive bacteria after being amidated, a process that enhances the positive charges on the altered protein structures ( **Pan *et al.*, 2007**). These impacts rely on the linkages between the antimicrobial peptide or protein and structural components of the bacterial membranes and cell wall **(Hancock, 2004)**.

The Shai–Matsuzaki–Huang model may allow for the early interactions of most antimicrobial peptides with membrane surfaces (**Shai, 1999**). In solution, the peptides are initially started as unstructured molecules. Upon contact with the membrane, the molecules acquire a three-dimensional shape (e.g., α-helix or β-sheet) that renders them amphiphilic, with the positively charged end engaging directly with the lipid headgroups. The peptide subsequently becomes embedded in the outer leaflet of the membrane, causing it to become thinner. X-ray diffraction and atomic force microscopy have recently confirmed this thinning **( Chen *et al.*, 2003)**.

After this phase, channel development is possible, but this aspect of the method is more contentious. Several theories, including the toroidal pore model, the carpet model, the barrel-stave model, and the micellar aggregate channel model, have been suggested to account for this phenomenon (**Wu *et al.*, 1999)**. Each model's appropriateness is contingent on the peptide (**Buffy *et al.*, 2004**) and lipid properties (i.e., elasticity, phase, hydration, and hydrophobic chain length) (**Dave *et al.*, 2005*)***.

Ultimately, the bacterial cells are eliminated in a variety of methods, including (i) depolarization of membranes (**Westerhoff *et al.*, 1989**), (ii) destruction of intracellular activities like macromolecular formation (**Kragol *et al.*, 2001**), (iii) degradation of cell walls (**Bierbaum and Sahl, 1985)**, (iv) alteration of membrane bilayer lipid content **(Matsuzaki, 1999),** and (v) the creation of micelles, which may cause cell leaking if circumstances are severe enough (**Papo and Shai, 2005**).

The biological activity of these proteins is frequently mediated through the creation of temporary DNA complexes. Protein-nucleic acid interactions may affect harmful microbes' physiological functions connected with DNA or RNA, promoting infection resistance. Broad bean protein isolate (BP), soybean protein isolate (SP), and chickpea protein isolate (CP) were esterified to boost their net positive charges. The antibacterial effect of the recombinant proteins was quantified and compared to the antimicrobial activity of the equivalent natural proteins.

**4.2. Glycinin and β-Conglycinin are two biologically active legume proteins:**

**I -Structure and Characterization:**

Because of its beneficial qualities, substantial nutritional value, and biological properties, soy protein is among the most significant vegetable protein sources. Approximately 70% of the storage proteins in soybean seed are composed of the two primary storage protein components, β-conglycinin (7S) and glycinin (11S) (**Mujoo et al., 2003**). Glycinin has six subunits, each comprising an acidic (A) and basic (B) polypeptide chain linked together by a disulfide bond. β-conglycinin is a non-disulfide-linked trimer comprising subunits numbered $\alpha^0$, α, and β **( Sitohy *et al.*, 2012)**.

Due to their high protein and other active constituent concentrations, soybeans benefit health (**Vasconcellos *et al.*, 2014**). Soybean protein isolates yielded glycinin, basic subunit, and β-conglycinin, which were compared to penicillin for their antibacterial activity towards infectious *Salmonella enterica* subsp. *enterica serovar Enteritidis*, *Listeria monocytogenes*, and spoilage bacteria (*Bacillus subtilis*). Evidence from an in-situ milk system demonstrated the efficacy of glycinin and its basic component as antibacterial agents towards spoilage and pathogenic microorganisms. This potency was linked to the basicity of the protein proportion, and these compounds have the potential to operate as effective and benign antibacterial agents in the preservation of food.

Milk may be recontaminated during preservation or usage, even after it has been pasteurized. Therefore, introducing this little amount of natural protein may prevent milk from contamination and improve its sanitation and preservation quality. Because of the external impact that glycinin and the basic component have on germs, they may be useful against microorganisms if they can be obtained by food or the digestive system. Due to their dual mechanisms of action, these compounds may be used alone and as an adjunct to other antibiotics (**Sitohy *et al.*, 2012)**.

**Li *et al.* (2015)** investigated the effects of glycinin basic peptide (GBP) on the membrane of Escherichia coli and found that it had antibacterial properties (**Li *et al.*, 2015**). Basic components, glycinin, and β-conglycinin were tested for their ability to inhibit the multiplication of spoilage and pathogenic microbes in pasteurized and raw milk **( Mahgoub *et al.*, 2016)**.

**4.3. Mechanism of action of modified natural proteins:**

Soybean protein esterification converts the negatively charged protein to a positively charged one, resulting in potent antimicrobial properties (**Sitohy *et al.*, 2013)**. This boosts the already strong positive charge on 11S while reversing the overall charge of 7S from negative to positive. The protein's antibacterial activity is restored by making this alteration since the previously inhibiting connection between the two subunits has been eliminated. In today's biotechnology, cationic proteins with antibacterial properties may be produced. These cationic protein combinations are ready to be used for antimicrobial purposes, eliminating the requirement for laborious and expensive methods of extracting the active protein constituent (11S).Cationic proteins' ability to inhibit microbial growth may begin with a combination of hydrophobic interaction between similar regions of the reacting species and an electrostatic attraction between the negatively charged regions of the cell membrane or the cell wall and the proteins' positively charged regions. MIC values for esterified soybean protein against *Salmonella enteritidis* and *Listeria monocytogenes* were reported to be 100 g/mL in a study conducted by **Sitohy *et al.* 2013** (**Sitohy *et al.*, 2013**).

Soybean proteins, both native and esterified, were tested for their ability to inhibit the proliferation of spoilage and pathogenic microorganisms in raw milk ( **Mahgoub *et al.*, 2011)**.

## V. INHIBITION OF PATHOGENIC BACTERIA BY NANOMATERIALS

Infections caused by bacteria are a leading source of both long-term disease and death. Due to their efficacy and low cost, antibiotics have become the standard therapy for bacterial illnesses. Nevertheless, numerous investigations have shown definitive proof that the overuse of antibiotics has resulted in the rise of bacteria resistant to multiple classes of antimicrobial drugs. Because of the widespread emergence and proliferation of antibiotic-resistant microbes, several researchers have studied how to manufacture potent antimicrobial agents capable of reversing or controlling this trend (**Mandal *et al.*, 2006**).

Many materials and methodologies have been applied like (i) nanomaterials (**El-Saadony *et al.* 2021)**, (ii) phage therapy (**Abdel-Shafi *et al*., 2020)**, (iii) herb extracts alone or in conjunction with conventional medications (**Abdel-Shafi *et al*., 2019**), (iv) probiotics ( **Abdel-Shafi *et al*., 2013**), and (v) plant or animal proteins **(Abdel-Shafi *et al.*, 2016).**

Particles between 1 and 100 nm in size are considered nanoparticles (NPs). There are notable differences between the chemical and physical characteristics of NPs and those of bulk materials. Even though NPs may be manufactured using a variety of chemical and physical processes, biological synthesizing is a green chemistry strategy that is safe, cost-effective, and easily compatible with living systems **(Gurkok and Ozdal, 2021)**.

Metallic nanoparticles have garnered much attention due to their potential as effective antibacterial compounds. Research into the antibacterial properties of metal NPs has been significant. These NPs include copper (Cu), silver (Ag), nickel (Ni), selenium (Se), gold (Au), titanium dioxide (TiO2), zinc oxide (ZnO), and iron oxide (Fe3O4) **(Hemeg, 2017).**

**5.1. Mechanisms of antibacterial properties of NPs:**

Unfortunately, the exact mechanism by which NPs exert their antibacterial effects remains unclear. One of the most well-known ways in which particles trigger cell death is through (i) inhibiting membrane permeability and bacterial cell respiration after binding to a bacterial cell, (ii) discharging harmful metal ions from NP surfaces, causing toxic effects, or (iii) producing reactive oxygen species (ROS) and triggering oxidative stress. Different types of NPs have different mechanisms. It is believed that metal oxide NPs, such as ZnO and TiO2, exert their antimicrobial properties primarily via the production of ROS (**Leung *et al*., 2016**). Metal ion release is believed to be the primary mechanism by which silver and gold inhibit the growth of bacteria (**Cui *et al*., 2012**).

**5.2. Synergistic effects of NPs with antibiotics:**

Antibiotic resistance may be combated, and the efficacy of antimicrobials improved by combining them with NPs. Moreover, they may minimize the requirement for and the harmful effects of antibiotic treatment **(Hutchings *et al*., 2019).** Antimicrobial resistance and cytotoxicity may be kept to a minimum by reducing antibiotic dosing when using nanoparticles in combination with effective medicines against bacteria. Soon, this synergistic impact of nanoparticles and antibiotics may be employed to combat harmful microorganisms.

In light of recent developments in nanotechnology, novel nanomaterials with many potent antibacterial characteristics may now be synthesized. The incorporation of nanoparticles into several substrates opens up a wide range of potential uses, from antibacterial synthetic fabrics to surgical and medical instruments **(Hemeg, 2017).**

**5.3. Nanoparticles as antimicrobial agents in NP-drug conjugate systems:**

NPs may be manufactured and mixed with other antibacterial drugs to increase their effectiveness against microbes with resistance. Their unique chemical composition allows nanoparticles to protect antibiotics from enzymes while facilitating their prolonged attachment to the target location. Consequently, increased antibiotic needs are minimized. To avoid infections caused by multidrug-resistant, harmful microorganisms, developing conjugates of antibiotic nanoparticles is crucial (**Jelinkova *et al*., 2019**).

Conjugated nanoparticles (NPs) are synthesized by combining chemical bonding with trans-cyclooctene, amine, hydrazide, sulfhydryl, isothiocyanate, and azide groups of drug and physical hydrophobic, host-guest, and electrostatic interactions **(Jelinkova *et al*., 2019)**. Numerous potent antibacterial nanoparticle-drug conjugate production mechanisms are discussed as follows:

- The antibiotic combination of nanoparticles has significant advantages in terms of the solubility of poorly soluble medications, the duration of the drugs' half-lives, their systemic circulation, and their release rates.

- Lipopolysaccharides, the negative charge of the peptidoglycan layer, and teichoic acid enhance NPs attachment and increase bacterial susceptibility to antimicrobial treatment.
- Adding hydrogen to NPs makes them more stable, but they lose their capacity to adhere to the surface with negative charges of bacterial cells, reducing their effectiveness.
- The NPs enhance the permeability of the bacterial cell membrane by attaching to proteins in the membrane, allowing more antibiotics to enter the bacterial cell.
- In addition to damaging membranes, disrupting protein-protein interactions, and causing metabolic problems, NPs are also toxic because of their active surface.
- NPs suppress respiration by interacting with sulfhydryl (-SH) groups in the cell wall, leading to cell destruction.
- Nanoparticles (NPs) that enter bacteria may disrupt cell membrane functioning (respiration and permeability).

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