Ministry of Higher Education & Scientific Research

Salahaddin University- Erbil

College of Agricultural Engineering Sciences

Department of Animal Resources

BACTERIOCINS AS MODERN ANTIMICROBIALS

Research Project

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1. INTRODUCTION

Decades of antibiotic misuse in clinical settings, animal feed, and within the food industry have led to a concerning rise in antibiotic-resistant bacteria (*et. al.*, 2021 Gradisteanu). Antimicrobial resistance (AMR) is alarming that the World Health Organization (WHO) just stated that combating AMR is one of the top 10 global health issues to track in 2021 by identifying its causes, routes of transmission, and encouraging the development of new antimicrobials (Almeida *et. al.*, 2021). Several factors contribute to the AMR crisis, the main drivers include antibiotics misuse and overuse by the general population and in food-producing animals, as well as their incorrect and indiscriminate administration by healthcare professionals (Prestinaci *et. al.*, 2015). For these reasons, a number of international health agencies (e.g., European Medicines Agency, Food and Drug Administration, World Health Organization, European Center for Disease prevention and Control) support the development of new antimicrobials and alternatives; such as bacteriocins, bacteriophages, probiotics, antibodies, and vaccines, have been investigated to reduce the use of antibiotics (Czaplewski *et. al.*, 2016 and Almeida *et. al.*, 2021).

Despite the use of modern food preservation techniques, the rate of food-related illness still increases and is a substantial cause of death, especially in countries where there is a lack of proper food safety monitoring systems. About one-third of the world population is suffering from food-related diseases each year due to the consumption of contaminated or intoxicated food like canned food, meat, poultry, and fermented dairy products (Akhtar *et. al.,* 2014).

Bacteriocins are produced by some group of bacteria and ribosomal synthesized peptides. Bacteriocins show inhibition activity to various groups of undesirable microorganisms. This compound is produced by Gram-negative, Gram-positive bacteria, and some archaebacteria (Surati, 2020).

Worryingly, it is estimated that by 2050 there will be no efficient antibiotic available to treat infections, if no new drugs are produced (Gradisteanu *et. al.,* 2021). Objectives of their studies have been focused on the potential, and challenges of using bacteriocins as natural bio-preservative agents. Moreover, identification and characterization of new bacteriocin, its approval process, and some related safety issue for the use of bacteriocin-producing bacteria as probiotics should be considered to ensure drug and food safety before marketing.

2. REVIEW OF LITERATURES:

2.1 Antibiotic-Resistant Bacteria (ARM)

The number of bacterial strains resistant to antibiotics has increased radically, making antimicrobial resistance (AMR) a major global health emergency of the 21st century. Currently, AMR infections are responsible for at least 700,000 deaths each year worldwide. If no action is taken, this figure could increase up to 10 million by the year 2050, with a global financial burden of USD

100 trillion. As a consequence of these malpractices, resistance to the so-called "last-resort" antibiotics is not an emerging problem anymore, but a reality (Almeida *et. al.*, 2021).

2.2 Control of microbial growth

Natural antimicrobials control microbial growth via disruption of cell structure and function. In the current review, such natural antimicrobials were classified based on their origin as shown in (Figure 1). Antimicrobials inhibit microbial growth, but do not eliminate the microbe. They act on specific microbial metabolic targets such as cell wall, cell membrane, genetic determinants or enzymes (Abdelhamid et. al., 2018). Different antimicrobials have different mechanisms to stop microbial growth (Pisoschi et al., 2018).

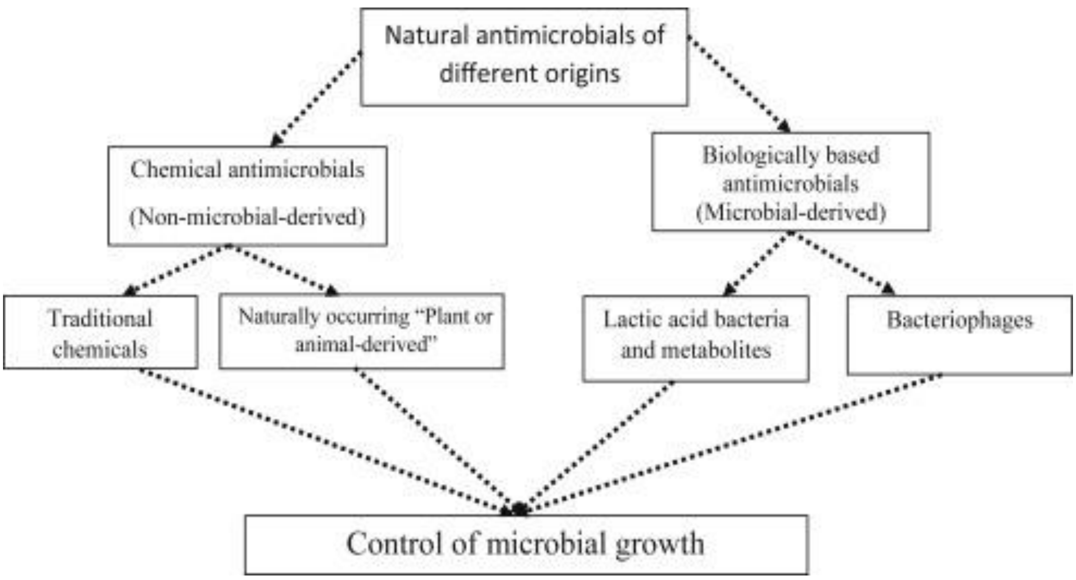

Figure 1. *Schematic representation of classifying natural antimicrobials which control microbial growth (Abdelhamid et. al., 2018)*

2.2.1 Chemical antimicrobials or "preservatives"

Chemical antimicrobials sometimes are known as preservatives. Chemical antimicrobials could be classified into two classes, “traditional" and “naturally occurring" (Davidson *et al.*, 2013). Traditional chemical antimicrobials have been used since many decades and approved by many countries. Sulfites, Nitrites, and Organic acids such as (benzoic, sorbic, acetic, lactic, and propionic) acids are commonly used as traditional food preservatives. Organic acids, in their undissociated form, pass through the cytoplasmic membrane lipid bilayer into the cytoplasm where they dissociated into anions and protons (Figure 2). Protons decrease the intracellular pH, and consequently inhibit glycolysis and active transport. Bacteria tend to exclude protons outside the cell on the expense of cellular energy. Thus, the antimicrobial effect of organic acids is likely attributed to such cascade of cellular events (Raybaudi-Massilia *et al.*, 2009) and (Abdelhamid *et. al.*, 2018). Traditional antimicrobials face some considerations because some could have risk to human health; nitrites link to cancer in young children and sulphite causes degradation of thiamin (V. B1) an essential vitamin, or affect food flavor (Garcia-Fuentes *et al.*, 2015).

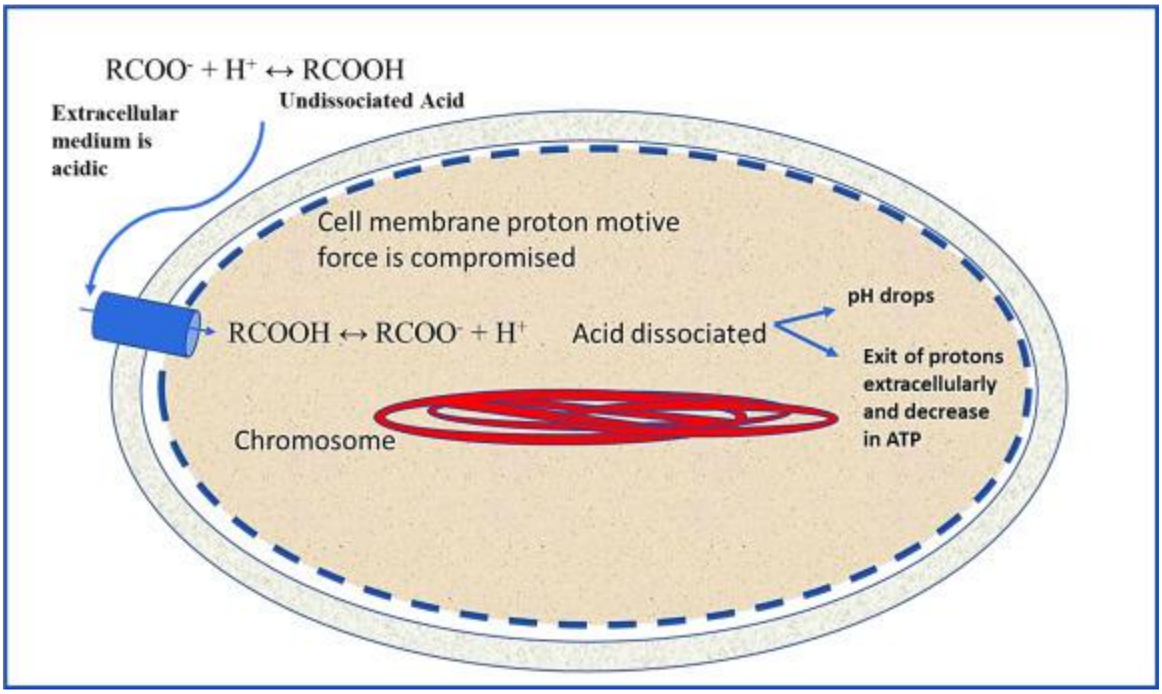

Figure 2. Mechanisms of the antimicrobial effect of "weak" organic acids against foodborne pathogenic bacteria (Abdelhamid *et. al.*, 2018).

2.2.2 Biologically-based antimicrobials

Antimicrobials of biological origin (mainly microbial) mostly used lactic acid bacteria (LAB), their metabolites or both to prevent the growth of undesirable microorganisms and improve the safety and quality of foods. LAB is considered a form of bio-preservation through food fermentation by controlled acidification. They are accepted by the consumers as natural bio-preservatives and health promoting microbes. The efficiency of lactic acid production, by LAB, depends on several factors such as food's fermentable carbohydrates, initial pH, and growth rate of LAB strains (Gobbetti and Di Cagno, 2017).

2.3. Modern Antimicrobials "Bacteriocins"

All living organisms produce antimicrobial proteins (AMPs), many of which are referred to as antimicrobial peptides because of their relatively small size. Within the eukaryotic immune system, AMPs are believed to serve as first line of defense in protecting the host against hostile intruders. Bacteria produce two types of AMPs: those that are ribosomally synthesized (also known as bacteriocins), and non-ribosomally synthesized AMPs, with no structural genes coding for these AMPs, e.g. e-poly-L-lysine (Chikindas et. *al*., 2018).

Bacteriocins, defined as ribosomally synthesized antimicrobial peptides, have traditionally been used as food preservatives, either added or produced by starter cultures during fermentation (Chikindas *et. al*., 2018). The bacteriocins have attracted many researchers and have been studied extensively. These small proteins or peptides (typically containing less than 60 amino acids) kill or inhibit the growth of some bacterial strains from similar or closely related species and usually have a narrow spectrum of bacterial growth inhibition. Bacteriocin-producing strains can be used as probiotics and food additives, in the treatment of pathogen-associated diseases and cancer therapy (Hassan *et. al.,* 2020).

Enterococcus spp. are one of the most frequent producers of bacteriocins (enterocins), which provides them with an advantage to compete in their natural environment, which is the gut of humans and many animals. Vancomycin-resistant Enterococcus (VRE), in particular, are an ongoing global challenge given the lack of therapeutic options. In this scenario, bacteriocins can offer a potential solution to this persistent threat, either alone or in combination with other antimicrobials (Almeida *et. al*., 2021).

Bacteriocins do not kill producer bacteria and are ribosomally synthesized. Their mode of action is thought to affect Gram-positive bacteria only, but some may antagonize Gram-negatives. Bacteriocin mechanism of action in most of the cases, cause cell membrane damage in target bacteria and their use is expanding in food safety. Bacteriocins cause conformational changes in the cytoplasmic membrane with pore creation (Figure 3), resulting in increased permeability and consequently leakage of ions and molecules from within the cell (Abdelhamid *et. al*., 2018). Some other examples of bacteriocins; Nisin has broad spectrum inhibitory activity against Gram-positive bacteria such as *staphylococci* and prevents spore germination in *Clostridium* and *Bacillus* (*Biswaro et al*., 2018*)*. Pediocin, a bacteriocin produced by *Pediococcus* spp., is also another example of bacteriocins with antagonistic potential against Gram-positive and some Gram-negative foodborne pathogens (*Ghosh et al., 2019*). Discovery of novel bacteriocins, similar to or more effective than nisin or pediocin, are a growing field in food safety research (Yang *et al*., 2016; Wu *et al*., 2019).

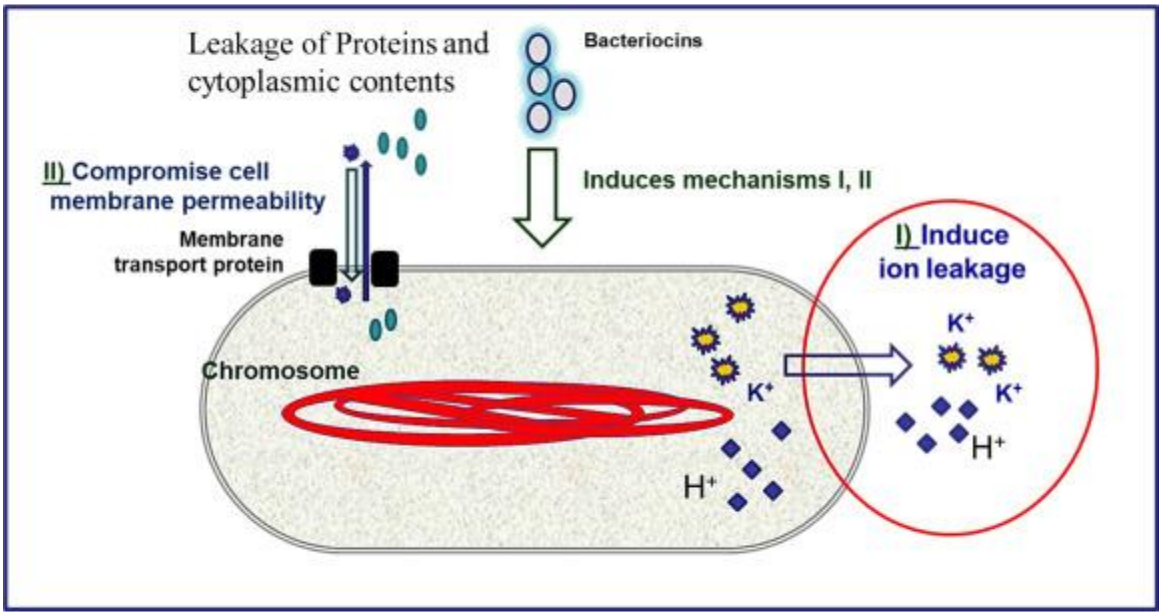

Figure 3. Mode of action of bacteriocins against foodborne pathogenic bacteria (Abdelhamid *et. al*., 2018).

2.4. Applications of Bacteriocins

Enterocins L50 (EntL50), were isolated from foodstuffs, has been identified either on the chromosome or plasmids of different E. faecium strains isolated from fermented foods. This is a broad-spectrum enterocin, composed by EntL50A and EntL50B peptides, that exerts antimicrobial activity against multiple Gram-positive and also Gram-negative bacteria, such as Escherichia coli, Salmonella enterica, Serratia marcescens, and Pseudomonas fluorescens (Almeida et. al., 2021).

Rationale for utilization of bacteriocins in livestock compared to antibiotics, most bacteriocins are relatively specific and can only affect a limited number of bacterial species. In the case of pathogens as target *organisms that colonize the gastrointestinal tract of poultry, cattle and swine, the use of bacteriocin-producing strains would have little effect on most beneficial intestinal bacteria. In poultry, the use of bacteriocin-producing bacteria (BPB) has been mainly targeted for the control Salmonella. (Diez-Gonzalez, 2007). Enteric fermentation in ruminants is the single largest anthropogenic source of agricultural methane and has a significant role in global warming. Consequently, innovative solutions to reduce methane emissions from livestock farming are required to ensure future sustainable food production (Doyle et. al., 2019). BPB capable of producing inhibitory bacteriocins against methanogenic bacteria could improve feed efficiency by reducing the amount of carbon lost in the form of methane. A mixture of Lactobacillus strains has shown promising results to reduce the fecal shedding of E. coli O157:H7 in cattle and is currently being marketed as a probiotic with the name of Bovamine (Diez-Gonzalez, 2007)*

3. Conclusions

Bacteriocins are one of many natural defense mechanisms bacteria use to compete against microorganisms in the same environment. Since the first discovery of nisin, many bacteriocins with unique structures and different modes of activity have been described. During the last decade, many investigators shifted their focus on bacteriocins for food preservation to the treatment of infections and antibiotic-resistant disease-causing bacteria. This exciting new era of bacteriocin research will undoubtedly lead to new inventions and new applications. With the rapid rate at which genome sequences are becoming available, genome mining becomes easier, and with the latest techniques in gene synthesis and protein expression, we can look forward to novel bacteriocins with very dedicated applications. Further research to study the properties, structure, and mode of action of such bacteriocins is required, especially for assessing their potential use in the biocontrol of bacterial diseases and as a substitute to antibiotics.

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