Future and role of bacteriocins as antimicrobial in food safety applications

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Background: Overuse and misuse of conventional antimicrobials has led to new strains of microbes with increasing levels of resistance, thus posing a top threat to the public's health and a priority across the globe. According to the National Institute for Health and Care Excellence (NICE), antimicrobial resistant (AMR) infections cause 700000 deaths worldwide per annum [1]. The estimated economic output loss as a result of AMR is1% of gross domestic product per year rising to 5% for developing counties by 2050 [2]. The additional cost to health systems is approximately US$1400 [3] per patient as a result of prolonged hospital stays and the need for more expensive and intensive care. Food sector is also affected due to this developing antimicrobial resistance. Recently, there is growing concern over the possibility of antimicrobial resistance transmission via food-borne pathogens. Development of new active, alternative drug therapies would reduce our reliance on conventional antibiotics that induce resistance in pathogens. In past decades, food industry has made use of varied chemicals to inhibit the growth of different food-borne microorganisms [4]. This triggered the food deterioration that further may have a detrimental effect on the sensory and organoleptic characteristics of some foods. To reduce the effect of chemical processing and preservation, bio-preservatives have been developed [5].

What are the concerns? To date, bacteriocins, produced by diverse bacteria, have shown a positive outcome as antimicrobial substitutes which have been successfully used as: (i) bio-preservative; (ii) anti-biofilm agents; and (iii) alternatives to the conventional antibiotics, to minimize the risk of emergence of resistant microorganisms. However, there are still several limitations including uncontrolled interactions of bacteriocins with various food components, its proteolytic degradation and electrostatic repulsion that challenge the use of these bacteriocins as food bio-preservatives. In the last years, several studies on bacteriocins have demonstrated that the optimization of their combinations with other antimicrobial agents, and the hurdle technology approach, could provide solutions to some of the challenges [6]. In recent era, the use of nanotechnology is a potential approach to overcome these limitations and to maximize the use of these peptides, nano-formulations are one of the most promising avenues.

The problem: In modern farming practice (aquaculture, livestock and crop production), antimicrobials are widely administered for therapy as such these are mass administered via feed or water. In some countries up to 80% of total antibiotic consumption is administered to animals which are not completely absorbed in the gut and excreted out with waste matter that is used to produce manure. This manure is used to fertilize soil resulting in transfer of antibiotic resistant genes (ARGs) to agricultural soil ecosystem and then onto plants [7]. The largest reservoirs of antibiotic-resistant bacteria (ARB) include fish, food animals and vegetables [8]. Furthermore, different antimicrobials are food preservatives which prevent food spoilage by suppressing the growth of food pathogens and thus accelerate AMR spread within and along the food chain.

The WHO estimates that 1 in 10 people fall ill from eating contaminated/spoiled food with 420 000 global deaths per year [9, 10]. Resistance of significantly concerning foodborne bacteria such as *Campylobacter, L. monocytogenes and Salmonella* to various conventional antibiotics. In particular there are insufficient drugs to target the WHO's priority Gram-negative pathogens, ESKAPE (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa and Enterobacter spp.*) [11].

The need: There is, therefore, an imminent necessity to prolong the lifespan of current antimicrobials or alternatives to conventional antibiotics and control the exposure of animals, crop, food and humans to antimicrobials in order to remain ahead of the AMR curve. One way of reducing exposure is through efficient and targeted delivery of antimicrobials to minimally processed food.

State of the Art: Antimicrobial compounds such as bacteriocin can be highly sensitive to the production process and/or storage conditions. Thereby, the antimicrobial efficacy of bacteriocin may be reduced because of undesirable interactions with food components and thus, large antimicrobial concentrations are often required. In recent era, the use of nanotechnology is a potential approach to overcome these limitations (to improve stability that prevents proteolytic degradation, dispersity, bioavailability of hydrophobic bacteriocins and functionality of bacteriocin, bacteriocin dose reduction which may extend the application of natural bacteriocin in foods) and to maximize the use of these peptides, nano-formulations are one of the most promising avenues [12]. The integration of nanotechnology and biotechnology opens the door to future perspectives to solve the problems belonging to a range of biological products [13]. Nano-formulation is a new field that has a growing role in food industry for safety measures. Different nano-formulation technologies have been favored by recent investigations to enhance the operations of natural antimicrobial substances, for better food functionality and quality. This has been achieved through the design of nano-encapsulating and nano-laminate delivery mechanisms that help in preserving sensory and organoleptic attributes of food [14]. Previous studies reported the metal nanoparticles of gold, silver, zinc, copper to solve the problem of antimicrobial resistance [15]. However, nanoparticles made from metals and inorganic sources had limitations like low rates of clearance, little scope for surface modification and enhanced toxicity effects [16]. Nano-carriers based on synthetic polymers like PLA and PLGA also exhibited limitations like aggregation of proteinaceous biomolecules encapsulated in their interior core [17]. Further, liposomes had poor drug encapsulation efficiency, low stability and high rates of release for biomolecules [18].

Have the concerns been addressed effectively? Nanotechnology may overcome this problem by encapsulation of antimicrobials into carbohydrate coated plant based protein nanoparticles, enhancing their efficacy and stability with specific targeting due to presence of polysaccharides onto the surface of proteins to combat antimicrobial resistance in food-borne pathogens. The use of polysaccharides in protein nanoparticles can be an alternative for the controlled release of antimicrobials [19]. Protein nanocomposites amalgamate the advantages of nano-sized structures with favorable characteristics of biomolecules. Proteins are biocompatible, biodegradable and abundant in nature. The amphiphilic nature and surface-active properties confer proteins their functional attributes, making them of great importance for the food industry as they can be applied into different products as emulsifiers, foaming or gelling agents [20].

Based on lower costs, health and religious, moral or environmental concerns related to the consumption of animal-based ingredients, Consumer trends towards plant-based foods and protein ingredients [21]. Therefore, nano-formulated bacteriocin encapsulated plant-based protein-polysaccharide conjugates could be a biodegradable and eco-friendly solution to tackle this challenge. However, during extraction, processing or storage, proteins can undergo partial or complete denaturation that can eventually affect their intended purpose and functionality. The different characteristics of proteins, such as their functional, surface and rheological properties, will directly impact on the sensory characteristics of food products, such as texture, aroma, flavor and appearance [22]. Nano-conjugation of proteins will improve the functional properties compared to those of the native protein. The solubility and other functional properties of plant proteins have been subsequently improved through the conjugation with carbohydrates [23].

Protein-carbohydrate conjugates can increase the thermal stability, and improved solubility, foaming, gelling and emulsifying properties, relative to the protein alone [24]. These improvements will broaden the use of proteins to products that require severe processing conditions (broader pH range, higher temperatures) and to the development of tailored protein- carbohydrate conjugates for specific uses. The impact of conjugation on the nutritional quality of the proteins, as well as other uses in the food and pharmaceutical sectors, such as antimicrobial, antioxidant and nano-encapsulation of compounds for delivery. A very few reports on encapsulation of antimicrobials into carbohydrate coated protein nanoparticles, for improved capabilities in the food and nutrition field for their application in delivery systems, packaging, food safety and security has been reported [25, 26]. Conjugation with protein–polysaccharide nanocomposite will enhance the antimicrobial activity of bacteriocins and, will also protect them from proteolytic degradation and extend shelf life of food product.

Which antimicrobials are currently deemed effective? Unlike free bacteriocins, nano-conjugated bacteriocins showed better stability and a wider range of antimicrobial action. Ultimately, nanotechnological approaches provide an attractive option for antimicrobial peptides to be developed on an industrial scale. Nisin is the most popular amphiphilic Bacteriocin/antimicrobial peptide, approved by the FDA, which has been used in the food sector for extending shelf-life and improving food safety [27]. Nisin has been used commonly in the food industry as an antibacterial agent against several food-borne Gram- positive strains such as *Staphylococcus aureus*, *Listeria monocytogenes, Leuconostoc, C. botulinum* and *C. sporogenes.* The widespread use of nisin with different nanoconjugates for bio-preservative applications has also led to the development of new packaging techniques with improved antimicrobial spectrum [28]. Nisin has been used as bio-preservatives in various foods, they are active against a range of food pathogenic and spoilage bacteria due to their active range of pH values and resistance to high temperatures. Nisin is classified as a class-I-a bacteriocin, also called lantibiotic and is the most commercially important and characterized bacteriocin. Pediocin is a class II-a bacteriocin that is produced by *Pediococcus spp*. Pediocin has been shown to be more effective than nisin against some food-borne pathogens such as *L. monocytogenes*, *S. aureus, Pseudomonas* and *E. coli* [29]. Lacticin is a two-component lantibiotic isolated from an Irish kefir grain used for making buttermilk. Lacticin exhibits antimicrobial activity against a wide range of food-borne pathogens and food spoilage bacteria in addition to other lactic acid bacteria (LAB) [30].

Futuristic scope: Microbial infections are very common (there are more than 1 million cases per year) in India one of the major complications leading to antimicrobial resistance. Antimicrobial resistance transmission via food-borne pathogens is a major concern all over the world. The government of India is very much aware of it and realizes that microbial infections are environmental as well as development issue. The Indian Public Health Authorities released their National Action Plan on Antimicrobial Resistance in 2018 at World Health Assembly in Geneva in May. The National Action Plan on Antimicrobial Resistance covers a wide range of themes and factors that drive antimicrobial resistance and clearly outlines all the challenges that need to be tackled. Therefore, government is fully committed to manage this issue so that country is protected from its adverse effects and the growth path remains stable. A set back is that the interaction mechanisms occurring in bacteriocin nanoconjugates responsible for strain inhibition is not fully understood as yet. Research in this field is in early stage and it is anticipated that progress can be made in the near future. To realize the true potential of bacteriocin- nanoconjugates for their application in food preservation, there is a need for understanding the interaction mechanisms between different bacteriocins and nanoconjugates and further, bacteriocin capsulated nanoconjugate interaction with pathogen cell wall. We are pretty hopeful that the carried scientific efforts in future will surely help to develop safe and effective bacteriocin nanoconjugate approach for food bio-preservation and food safety application which will in turn reduce the antimicrobial resistance problem which has to be solved as soon as possible to save mankind.

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