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Bacteriocins from lactic acid bacteria and their applications in meat and meat products

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ABSTRACT

Meat and meat products have always been an important part of human diet, and contain valuable nutrients for growth and health. Nevertheless, they are perishable and susceptible to microbial contamination, leading to an increased health risk for consumers as well as to the economic loss in meat industry. The utilization of bacteriocins produced by lactic acid bacteria (LAB) as a natural preservative has received a considerable attention. Inoculation of bacteriocin-producing LAB cell as starter or protective cultures is suitable for fermented meats, whilst the direct addition of bacteriocin as food additive is more preferable when live cells of LAB could not produce bacteriocin in the real meat system. The incorporation of bacteriocins in packaging is another way to improve meat safety to avoid direct addition of bacteriocin to meat. Utilization of bacteriocins can effectively contribute to food safety, especially when integrated into hurdle concepts. In this review, LAB bacteriocins and their applications in meat and meat products are revisited. The molecular structure and characteristics of bacteriocins recently discovered, as well as exemplary properties are also discussed.

1. Introduction: the need for natural antimicrobials in meat application

Microbial contamination causes serious safety and quality problems in meat industry. Meat and meat products, particularly fresh meat, contain adequate amount of water and abundance of proteins and essential nutrients with favorable pH for supporting microbial growth. The microorganisms present on meat and its products are in broad spectrum, ranging from bacteria to yeasts, molds and viruses, depending on type of the products. By far, microbial issues in meat industry have arisen mostly due to bacteria (Hui, 2012). As reviewed by Jayasena and Jo (2013), the main spoilage bacteria in meat include *Pseudomonas*, *Acinetobacter*, *Brochothrix thermosphacta*, *Moraxella*, *Enterobacter*, *Lactobacillus*, *Leuconostoc*, and *Proteus*. Upon a substantial growth of those spoilage organisms, proteins and lipids of meat and meat products undergo degradation, adversely changing appearance, texture and flavor of the products (Borch, Kant-Muermans, & Blixt, 1996). Normally, spoilage microbes do not harmfully affect health but they can stimulate gastrointestinal disturbances when consumed in high concentrations (Jayasena & Jo, 2013).

In addition to microbial spoilage, meat and its products are also prone to contamination by pathogenic microorganisms. Nine major pathogenic bacteria associated with meat and meat products include *Salmonella* spp., thermophilic *Campylobacter jejuni*, enterohemorrhagic *Escherichia coli* O157:H7, *Clostridium perfringens*, anaerobic *Clostridium botulinum*, *Listeria monocytogenes*, *Staphylococcus aureus*, *Bacillus cereus*, and *Yesinia enterocolitica*, causing illness or even death in humans (Hui, 2012). The outbreaks caused by contamination of those pathogens in meat are steadily occurred. An example of the recent foodborne outbreak results from the contamination of *Salmonella* in pork reported during April to September 2015 from five states along the West Coast of the US (Center of Disease Control and Prevention, 2015), leading to a major recall of more than 520,000 pounds of pork from the responsible company (Johnston, 2015). This multistate outbreak resulted in a total of 192 ill people, 30 were hospitalized. Luckily, no death was reported in this case. The incidence, however, instantly became headlines in global-wide media, raising consumer suspicion in safety of meat and its products.

Application of physical and chemical technologies has been employed to inactivate microbes in meat and meat products. Physical processes, such as freezing, refrigerating or thermal processing, however, may not completely assure safety of meat and its products and meet consumer satisfaction. Antimicrobial agents have been widely used. Incorporation of antimicrobial compounds with non-thermal

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processing is of interest as this alternative hurdle technique can enhance microbial inactivation and allow preservation of desirable characteristics of most foods (Ross, Griffiths, Mittal, & Deeth, 2003). The active compounds provide preventive effects during the processing as well as against post-process microbial contamination, extending shelf life of the products. According to an increased negative perception towards chemical agents, natural antimicrobial agents have been extensively screened and tested for their effectiveness in foods. The approved antimicrobial agents in meat, their usages and challenges are discussed in the next section.

2. Antimicrobial agents used in meat and meat products

Antimicrobial agents have long been directly applied as food additives or used as processing aids with primary intention of prolonging shelf life and preserving quality of meat and meat products. In general, food additives refer to substances not consumed as foods but intentionally added to a food, and it or its by-products become components of the food. Processing aids are also substances that are not normally consumed as food by itself but intentionally used in the processing of raw materials, foods or their ingredients, for a certain technological purpose during treatment or processing. Application of processing aids may result in the unintentional but technically unavoidable presence of residues or its derivatives provided in the final product. Nevertheless, the resulting residues or derivatives do not have any technological effect on the final product. The use of antimicrobial agents in meat and meat products has been approved in 21 Code of Federal Regulations for use in meat, poultry, and egg products as food additives (USFDA, 2015). For European Union (EU), the use of food additives in meat preparations is stated in the Commission Regulation (EU) No. 601/2014 (The European Commission, 2014).

Addition of nitrites, organic acids (i.e. lactic, ascorbic, benzoic and sorbic acids) and its salts as food additives in meat and meat products are approved by Codex Committee on Food Additives as stated in General Standard for Food Additives (GSFA). Blends of propionic acid, caprylic acid and acetic acid are generally used in marinated meat (Smith, 2012). Although sorbic acid/sorbates are not allowed in meat and meat products as specified by GSFA, the solution of 10% potassium sorbate may be applied to unrefrigerated dry sausages in the US (Stopforth, Sofos, & Busta, 2005). Application of organic acids is limited due to their potential negative impact on flavor and color of the products (Smith, 2012).

Organic acids, inorganic phosphates and oxidizers, are also applied as processing aids for quality control. Those chemical antimicrobial agents can be used in combination with hot water and steam treatments for carcass and fresh meat decontamination (Simpson & Sofos, 2009). In the US, the use of antimicrobials for such applications is approved by the Food Safety and Inspection Service (FSIS), if the chemicals (i) are generally recognized as safe (GRAS), (ii) do not lead to adulteration, (iii) do not create labeling issues, (iv) are scientifically proven to be efficacious, and (v) do not pose human health issues to worker or consumer (Koutsoumanis, Geornaras, & Sofos, 2006; Simpson & Sofos, 2009). According to the EU regulation (EU) No 101/2013, beef carcass can be treated with lactic acid to reduce microbiological surface contamination (The European Commission, 2013).

Today, the application of chemical preservatives has been questioned because of the potential toxic and carcinogenic effects (Sax & Lewis, 1989; Schaubschläger, Becker, Schade, Zabel, & Schlaak, 1991; Tompkin, 2005). Not only pressure of the consumer health concerns, but also a trend towards natural food additives so called "clean-labeling" has driven exploring of natural antimicrobial compounds as an alternative to synthetic food additives (Castellano, Belfiore, Fadda, & Vignolo, 2008; Deegan, Cotter, Hill, & Ross, 2006).

Among natural antimicrobial compounds, numbers of plant-derived extracts have been widely studied (Cowan, 1999; Lucera, Costa, Conte, & Del Nobile, 2012).The phytochemicals are plant secondary metabolites playing roles in defense mechanisms against microbes and predators as well as contributing to flavor and aroma (Cowan, 1999). The plant-based antimicrobial substances can be divided into groups of phenolics, terpenoids (essential oils), alkaloids, lectins and polyacetylenes (Cowan, 1999). Extracts of spices and herbs commonly used in foods receive tremendous attention. In meat and meat products, the plant extract can be used alone or combined with the other extracts or with a minimal process for synergistic output. Recent studies, for example, indicated inhibitory effects of clove and cinnamon oils in ground chicken against *L. monocytogenes* (Hoque, Bari, Juneja, & Kawamoto, 2008), thyme and balm oils in fresh chicken breast (Fratianni et al., 2010) and hop extracts in marinated pork (Kramer et al., 2015) against broad-spectrum of meat spoilage bacteria. Phytochemicals are certified as GRAS (Lucera et al., 2012), and pleasantly accepted by majority of consumers in comparison with synthetic preservatives. It is worth noting that properties of meat and process condition can significantly interfere with the antimicrobial efficacy of the plant antimicrobials. Compared to *in vitro* assays, a greater concentration of phytochemicals is required to achieve the same effect in food (Jayasena & Jo, 2013; Kramer et al., 2015; Sultanbawa, 2011). In addition to their strong flavor, usage of the phytochemicals at high concentration is subjected to critical scrutiny on the safety due to limited toxicological information (Sultanbawa, 2011).

Advancement in protein and peptide research has led to discoveries of natural antimicrobial proteins and peptides. To date, lysozyme, lactoferrin, and nisin are the only three proteins and peptides approved for application in meat and meat products (Davidson & Branen, 2005). Lysozymes, a natural lytic enzyme present in egg white, possess capability to hydrolyze the glycosidic bonds linking peptidoglycans, causing the leakage of bacterial cell wall (Losso, Nakai, & Charter, 2000). The allowable usage of egg-white lysozyme is 2.5 mg/lb of sausage casings or 2.0 mg/lb of ready-to-eat meat or poultry products (USFDA, 2000a). As for lactoferrin, the milk-derived iron-binding glycoprotein competes with the iron acquisition of bacteria, inhibiting bacterial growth. Lactoferrin can block microbial adhesion on biosurfaces by disrupting outer-membrane proteins of Gram-negative pathogens, preventing microbial colonization on the meat surfaces (Atef Yekta et al., 2010; Naidu, 2002). Low concentration of lactoferrin was shown to detach viable or dead tissue-bound bacterial cells (Naidu, 2002). Although milk-derived lactoferrin is considered as GRAS, the ingredient must be labeled when beef carcasses and cuts are treated with water-based spray containing up to 2% milk-derived lactoferrin. Thereby, milk-allergic individuals will be aware of the presence of milk-based ingredient. No labeling is required when the lactoferrin treatment is followed by sufficient rinse to leave a residual concentration lower than 800 ppb (USFDA, 2003). While lysozyme effectively inhibits gram-positive bacteria, lactoferrin exhibits broad-range antibacterial and antiviral activities (Naidu, 2002). Similar challenges of lysozyme and lactoferrin are the reduced efficacy when directly added into food matrices. Denaturation induced under harsh processing conditions also decreases the antimicrobial effect.

In contrast to plant extracts and the other protein-based antimicrobial preservatives, bacteriocins tolerate high thermal stress, are active over a wide pH range and remain effective at fairly low concentration (Cleveland, Montville, Nes, & Chikindas, 2001). Application of the bactericidal peptides does not alter sensory quality of food products, as the peptides present colorless, odorless, and tasteless characteristics. Based upon their advantageous characteristics, bacteriocins have been attracting considerable interest as an alternative natural food preservative to extend shelf life and safety of meat and meat products. The main objective of this review is to update current status of bacteriocins and their application in meat and meat products. Novel bacteriocins discovered in our laboratory, together with their structures, characteristics, as well as exemplary properties, will also be addressed herein.