Enterocin as a Biopreservative for Raw Meat Products

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Food preservation is a method used to handle and treat food products to slow down food spoilage and subsequently reduce the risk of foodborne illness. Nowadays, the demand for natural preservatives over chemical preservatives in food is increasing due to the awareness of consuming healthy food products without the risk of harmful side effects. Thus, the research and development of preservation techniques, referred to as biopreservation, is growing rapidly. In biopreservation methods, microorganisms that are known as lactic acid bacteria (LAB) and their antimicrobial substances are used to extend shelf life and maintain the nutritional value of foods. Among the most studied LAB are from the genus *Enterococcus*, which produces a bacteriocin called enterocin. Bacteriocins are ribosomal-synthesized antimicrobial peptides that are capable of inhibiting the growth of pathogenic bacteria that cause spoilage in food. LAB is generally regarded as safe (GRAS) for human consumption.

Keywords: food preservation ; natural preservatives ; lactic acid bacteria ; antimicrobial substances ; enterocin ; commercial techniques

1. Preservative Effects of Bacteriocin Produced by Lactic Acid Bacteria on Raw Meat Products

LAB is known for its capability to produce a variety of antimicrobial agents that can inhibit the growth of pathogenic bacteria. In 1988, the FDA approved the use of nisin and pediocin, a bacteriocin produced from *Lactococcus lactis* and *Pediococcus* sp. as preservatives for application in the food industry. Nisin and pediocin have been successfully commercialized widely [1]. In addition to nisin and pediocin, a bacteriocin from *Enterococcus* sp. namely enterocin has also gained significant academic interest following the research conducted on the effectiveness of antimicrobial agents produced by this species for use in food as a preservative. Ben Braïek et al. [2] stated that enterocin produced by *Enterococcus* sp. has high anti-listerial properties due to the bacteriocins produced by *Enterococcus* species being mostly classified as class III. It has a C-terminal disulfide bridge that stabilizes the posterior fold in the structure, which is crucial in enhancing the antimicrobial activity of the species [3]. In a study conducted by Fathizadeh et al. [4], recombinant bacteriocin, enterocin A and colicin E1 (ent A-col E1) exhibited antibacterial characteristics against both Gram positive and negative bacteria. Enterocin 12a produced by *E. faecium* was able to inhibit the growth of pathogens, such as *Salmonella enterica, Shigella flexneri, Vibrio cholerae, E. coli* and *L. monocytogenes* [5]. Several studies have reported the effectiveness of bacteriocin produced by LAB in inhibiting the growth of *L.monocytogenes* as shown in (**Table 1**). LAB are mainly from the genus of *Enterococcus* (*E. lactis Q1, E. lactis 4CP3, E. faecalis*), *Lactobacillus* (*L. paracasei, L. plantarum, L. sakei, L. reuteri*), and *Pediococcus*. Most of the bacteriocins produced by these LAB were able to inhibit the growth of *L. monocytogenes*. Based on **Table 1**, the treatment of *E. lactis* 4CP3 (enterocin A, B, P), and *E. faecalis* (enterocin AS-48) against *L. monocytogenes* resulted in growth inhibition activity as reported by Ben Braïek et al. [2][6] and Sparo et al. [7]. Meanwhile, enterocin P produced by *E. lactis* Q1 reportedly exhibited antimicrobial activity, as observed in the stunted growth of *L. monocytogenes* after 7 days of treatment as compared to the untreated sample [8]. Paracin C by *Lactobacillus paracasei*, Plantaricin (EF, W, JK, S) produced by *Lactobacillus plantarum* and bacteriocins produced by *Lactobacillus sakei*, *L. reuteri, L. plantarum, L. fermentum* inhibited the growth of *L. monocytogenes* while the treatment of Sakacin G produced by *Lactobacillus sakei* resulted in a decrease in the number of *L. monocytogenes* cells on roasted meat [9]. In addition, pediocin produced by *Pediococcus* sp. was found to exert broad spectrum antimicrobial activity against *L. monocytogenes* [10].

Table 1. Bacteriocin produced by lactic acid bacteria tested on raw meat.

Lactic Acid Bacteria	Bacteriocin	Inhibitory Effect	References
***Enterococcus lactis* Q1**	**Enterocin P**	***L. monocytogenes* cell decreased to 6.47 ± 0.30 log unit after 7 days as compared to control that was not treated with *E. lactis* (7.25 ± 0.35 log unit after 14 days) and maintained until 28 days in the fridge.**	**[8]**
***Enterococcus lactis* 4CP3**	**Enterocin A, B, and P**	**The growth of listerial was completely inhibited from day 14 until 28.** **The inhibition of *L. monocytogenes* growth on the rabbit meat during cold storage was detected on day 28.**	**[2][6]**
Enterococcus faecalis	**Enterocin AS-48**	**There was no detection of *L. monocytogenes* growth on the beef after 24 h treated with *E. faecalis*.**	**[7]**

Lactic Acid Bacteria	Bacteriocin	Inhibitory Effect	References
Lactobacillus paracasei	**Paracin C**	**The growth of pathogenic bacteria was inhibited, and the color of the meat was retained until day 15.**	[11]
Lactobacillus plantarum	**Plantaricin EF, W, JK and S**	**The growth of both spoilage bacteria was inhibited by *L. plantarum* until day 15 at 22 °C.**	[12]
Lactobacillus sakei	**Sakacin G**	**The application of *L.sakei takes* on roasted meat resulted in a decrease in the number of *L. monocytogenes* cells. Meanwhile, for chicken breast, the inhibition effect depleted.**	[9]
Lactobacillus sakei, L. reuteri, L. plantarum, L. fermentum	**Bacteriocins**	**The formation of the inhibition zone after the treatment of bacteriocin demonstrated the growth inhibition of *L. monocytogenes*.**	[13]
***Pediococcus* sp.**	**Pediocin**	**Pediocin and pediocin-like bacteriocins exerted a broad spectrum of activity against *L. monocytogenes* through the formation of pores in the cytoplasmic membrane and cell membrane dysfunction.**	[10]

2. The Application of Enterocin on Raw Meat Products

Nowadays, the preservation methods in the food industry are evolving, with the use of bacteriocin aiding the process of preserving raw products. Bacteriocin is known for its capability to inhibit the growth of spoilage bacteria, such as *L. monocytogenes*, *Salmonella sp*., and *E. coli* in commercial food products so the quality can be maintained over a certain period. A newly reported byproduct rich in enterocin AS-48, and known to have a wide spectrum of antibacterial activity, might have good potential to be used as an additive since it achieved a good safety profile indicated by the negative result of the mutagenicity and genotoxicity assay test [14]. About 500 µL/animal/d of enterocin have been used as additives and were administrated in the drinking water of rabbits. As a result, the enterocin significantly affected the quality and mineral content of the rabbit meat, mainly iron and phosphorus [15]. There are several species of *Enterococcus* used as preservatives in raw products. For instance, the cell-free supernatant of *Enterococcus faecium* TJUQ1 combined with the bacterial cellulose of *Gluconabacter xylinus* forms a composite film, BC-E, which shows antibacterial activity against *L. monocytogenes* after being soaked and applied on ground meat [16]. Other examples were recorded as shown in (**Table 2**). There are several techniques for incorporating bacteriocin into food products: (1) inoculation of bacteria producing bacteriocin directly onto the meat or meat products as a starter or protective culture, (2) the use of purified or semi-purified cell-free supernatant directly as a food preservative, and (3) incorporation of purified and semi-purified bacteriocin and in packaging material [17][18].

Table 2. The types of enterocin produced by *Enterococcus* sp. used in raw meat products.

Producer Strain	Types of Enterocin	Product	Additional Technique Used	Targeted Pathogenic Bacteria	References
E. faecalis	**Enterocin As-48**	**Fermented sausage**	**Mixed with bacteriocin/chemical preservatives**	***L. monocytogenes***	[18][19]
E. durans	**Enterocin L50A-like bacteriocin & L50B (Dur 152A)**	**Ham**	**Semi-purified bacteriocin/anti-listerial protection**	***L. monocytogenes***	[20][21][22]
E. faecium	**Enterocin A and B**	**Fermented dried sausage, minced pork, and ham**	**Applied on the surface of meat/alginate film/high hydrostatic pressure**	***Listeria spp, L. sakei***	[18][23][24][25]
E. classeliflavus	**Enterocin 416kk1**	**Cacciatore (Italian sausage)**	**Starter culture/low-density polyethylene film**	***L. monocytogenes***	[26][27]
E. mundtii	**Mundticin**	**Fermented fish and seafood, sausage**	**Starter culture/chitosan**	***L. monocytogenes***	[1][28]

Abts et al. [29] stated that enterocin is used as a food preservative through two methods: (1) direct inoculation of bacteria producing enterocin directly as a starter or protective culture, and (2) the use of purified or semi-purified cell-free supernatant. However, enterocin is often widely applied as a starter culture. For example, *E. faecium*, *E. mundtii*, and *E. classeliflavus* have been used as a starter culture in the production of fermented sausage [1][30][26]. As a result, *Enterococcus* sp. competes partially during the meat fermentation process, inhibiting the growth of *Listeria* sp. in the product [30].

Enterocin is also associated with several biochemical activities that stimulate aroma development through glycolysis, proteolysis, and lipolysis activities. In addition, it also plays a role in reducing the activity of metmyoglobin (MetMbO), which is an important mechanism for maintaining meat color [31]. Furthermore, enterocin also helps the degradation of

stachyose and raffinose, the non-digestive oligosaccharides known as anti-nutrient factors [32]. The use of purified or semi-purified cell-free supernatant is also one of the methods often used for raw products, conferring the same benefits as that of the inoculation method in terms of inhibiting the growth of *L. monocytogenes*. This method is particularly useful in stimulating the formation of compounds that give aroma and taste to the product. However, this preservation method also has several disadvantages. While bacteriocin can inhibit oxidative rancidity due to damage that occurs in fats or oils, the production of unwanted flavors may also occur as a result of fat hydrolysis by lipase enzymes or from contaminating microorganisms [17].

Several researchers have suggested that the use of purified or semi-purified cell-free supernatants is suitable for application in food products, as it is more effective than the direct inoculation of the bacteriocin-producing bacteria. The latter may cause damage to the food in hostile environments [33]. During the purification process, all contaminants with low molecular weight are removed, leaving only the bacteriocin with a specific activity. The purification step allows for a more accurate determination of the biological activity of bacteriocin [29]. On the other hand, it has been reported in some cases that the use of cell-free supernatant on raw meat can potentially reduce the antimicrobial activity of bacteriocin due to the protein degradation that takes place when the supernatant is absorbed into the meat matrix [17]. Thus, Silva et al. [34] and, Borges and Teixeir [35] have suggested an alternative method by incorporating the purified or semi-purified bacteriocin in packaging material to increase the activity and stability of the bacteriocin in complex food systems. Referring to **Table 2**, enterocin A and B from E. faecium were incorporated into an alginate film, which is one of the packaging techniques used for fermented dried sausages, minced pork, and ham [23].

3. Effects of Enzyme, Temperature, and pH on the Activity of Enterocin

Characterization of bacteriocin is important to evaluate its effectiveness to be applied in the food industry. According to previous researchers, *E. faecalis* and *E. faecium* are the most commonly used bacteria from the genus *Enterococcus*, particularly in the food industry. The bacteria are used for the preservation of raw materials due to their high stability against extreme temperature and pH as compared to other species of *Enterococcus* as shown in (**Table 3**). The sensitivity of bacteriocin towards pH is diverse. The bacteriocin, known as enterocin As-48 produced by *E. faecalis* maintains its activity at pHs as high as 12 and temperatures of 121 °C for 15 min. Meanwhile, the activity of bacteriocin produced by *E. lactis* and *E. durans* was inhibited at 121 °C after 15 min. On the other hand, *E. mundtii*, which produces mundticin, can maintain its stability at 121 °C for 15 min; however, its activity is typically inhibited at pH 12. By referring to **Table 3**, *E. faecalis* and *E. faecium* are suitable for application on food products, such as raw meats and vegetables since they are stable at high temperatures and pH.

Table 3. Activity of bacteriocin produced by *Enterococcus* sp. against enzymes, temperature, and pH.

Strain	Bacteriocin	Stability															
		Enzyme					Temperature (°C/min)				pH						
		Proteases K	Trypsin	Chymotrypsin	Lipase	Catalase	65 °C/30 min	80 °C/30 min	100 °C/30 min	121 °C/15 min	2	4	6	8	10	12	
E. faecalis	Enterocin As-48	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	
E. faecium	Enterocin A and B	−	−	+	+	+	+	+	+	−	+	+	+	+	+	+	
E. durans	Enterocin L50A- like bacteriocin and L50B, Durancin GL	−	−	+	+	+	+	+	+	−	+	+	+	+	−	−	
E. mundtii	Mudticin	−	−	+	+	+	+	+	+	+	+	+	+	+	+	−	
E. lactis	Enterocin A, B, and P	−	−	+	+	+	+	+	+	−	+	+	+	+	+	−	

Bacteriocin produced by *Enterococcus* sp. as listed in **Table 3** is typically sensitive to proteolytic enzymes, such as protease K and trypsin, which demonstrated the proteinaceous properties of the bacteria. Meanwhile, chymotrypsin, lipase, and catalase do not exert any effect on enterocin activity, indicating that the inhibition of bacterial growth is not due to the production of hydrogen peroxide [37]. Application of this proteolytic enzyme leads to protein degradation, and therefore, is safe for human consumption [32]. In the meantime, the loss of activity of bacteriocin depends on the formation of peptides and amino acid sequences.

According to Gao et al. [38] lowering the pH will gradually deactivate the growth of microorganisms. Most cationic bacteria will undergo cell lysis as a result of stimuli formed by negatively charged molecules found on the bacterial cell surface,

such as lipopolysaccharide (LPS), lipoteichoic and teichoic acids. The findings demonstrate that the bacteriocin produced by *Enterococcus* sp. has a high resistance to extreme pH ranges and has the potential to be used in acidic and alkaline processed foods [39].

Temperature is crucial in ensuring the stability of bacteriocin activity. Based on **Table 3**, the activity of enterocin from *E. faecalis*, and *E. mundtii* is stable at a maximum temperature of 121 °C for 15 min while *E. faecium*, *E. durans*, and *E. lactis* could only withstand temperatures up to 100 °C for 30 min. Enterocin produced by *Enterococcus* is a heat-tolerant bacteriocin. The activity performed differs according to the species and molecular structure of the respective bacteriocin [19][39]. Some highly heat-sensitive bacteriocin lose their activity at 50 °C due to the loss of their original secondary and tertiary structure as a result of denaturation [40]. The resistance of *Enterococcus* at pasteurization temperature and its adaptability to substrate and growth conditions demonstrates its potential application in food products.

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