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Structure, mode of action and application of pediocin natural antimicrobial food preservative: A review

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Abstract: Pediocin is a low molecular weight (2.7-17 KDa) cationic molecules compose of a hydrophilic N-terminal part containing the pediocin box (YGNGV) motif and a hydrophobic or amphiphilic C-terminal variable part which is produced by some of *Pediococcus* bacteria. Pediocin has inhibition properties against several sensitive bacterial cells and acts on the cytoplasmic membrane during pores formation. Pediocin intervenes with the absorption of amino acids in phospholipids of cytoplasmic membrane from target cells. The pediocin molecule has unique properties such as the thermostability and detaining of activity at a wide pH values and resistance to some proteases enzymes. Pediocin has inhibition activity against many food spoilage bacteria and pathogenic bacteria including several bacterial genera such as *Listeria*, *Clostridium*, *Bacillus*, *Aeromonas, Staphylocoocus, Lactobacillus* and *Enterococcus.* This review will assist in learning the challenges that yet require to attention to apply pediocin in food industries as food preservative.

Keywords: Food preservative, *Pediococus*, Bacteriocin, Pediocin, antimicrobial.

Introduction

Bacteriocins are antimicrobial peptides or toxins peptides which produced from bacteria in end stationary phase or death phase to inhibit bacterial species which are genetically closely related. Some species of yeasts and *Paramecium* had been inhibited by killing factors (bacteriocins) (Cotter *et al.*, 2013). Some yeasts such as *Saccharomyces boulardii* can peptides produce like bacteriocins which is called protein killer (Niamah *et al.*, 2017b, c).

The produced bacteriocins from Gram positive bacteria such as lactic acid bacteria are classified into four classes depending on their molecular weight, heat resistance and the presence of modified amino acids (Cotter *et al.*, 2005; Niamah, 2010; Perez *et al.*, 2014). Class I: called is lantibiotics which contains modified amino acids after DNA translation. Nisin is the most exclusive component of class I (Jung, 1991). Class II: defined as peptide of small molecular weight (<10 kDa), heat-stable, non-modified amino acids and that shows strong antilisterial activity. Pediocin is the most characterized component of the class II (Eijsink *et al.*, 2002; Rodríguez *et al*., 2002). Class III: Large molecular weight (>30 kDa), unstable heat proteins, predominantly it is produced by enzymatic activity (Nilsen *et al*., 2003). Class IV: complex or globular proteins, composed of peptides with carbohydrate or lipid (Maqueda *et al*., 2004; Gabrielsen *et al*., 2014).

Pediocin is one of class IIa group. It is produced from *Pediococcus* spp. The *Pediococcus* genus is Gram-positive, homofermentative and bellows to Lactobacillaceae family (Haakensen *et al*., 2009). *Pediococcus* genus includes several species such as *P. acidilactici, P. pentosaceus, P. damnosus, P. cellicola, P. parvulus, P. stilesii, P. inopinatus, P. claussenii* and *P. ethanolidurans* (Franz *et al.,* 2006). The production of bacteriocin from these species is called pediocin. Some studies added symbols with Pediocin word such as pediocin AcH (Bhunia *et al*.,1988), pediocin SJ-1 (Schved *et al*.,1993), pediocin JD (Christensen and Hutkins, 1994), PA-1 (Rodríguez *et al*., 2002) and pediocin PaF-11(Marwati *et al*., 2017).

Pediocin activity in foods is affected by many factors such as pH, heat treatment, proteolytic enzymes, salts and storage time (Niamah, 2010). The mode of action of pediocin is making pores in the cytoplasm of the targeted cells then reducing the intracellular pH and inhibits protein stimulation which is responsible for energy production in the cell (Bhunia *et al*., 1991; Gabrielsen *et al*., 2014).

Nisin and pediocin have been used as food preservatives like fermented sausage (Woraprayote *et al*., 2016; Castellano *et al*., 2017), fermented vegetables and dairy products (Verma *et al*., 2017), these bacteriocins, added bacteria starters are possibly produced during fermentation or may be added pure bacteriocin to food. In this review, we will research on the full papers and published literatures of pediocins. Topics such as pediocin structure, action mode of pediocin, spectrum of pediocin activity and pediocin applications in foods will be discussed in detail.

Pediocin structure

Pediocin peptide consists of 40-44 amino acids, its molecular structure has 4 molecules of lysine, 3 molecules of histidine, one molecule of aspartic acid and 4 molecules of cysteine which linked by disulfide bonds in site (C9-C14) and (C24-C44) of pediocin structure (Chikindas *et al*.,1993).

The charge of pediocin is positive between (+3) and (+7) when pH=6 while the isoelectric point (Ip) is 8.6-10 (Venema *et al.,* 1997). The N-terminus of pediocin contains hydrophilic amino acids and β-sheet structure. The C-terminus of pediocin contains amphiphilic amino acids and α-helical structure. The location of pediocin box in N-terminus of pediocin structure (Fig 1).

The calculated molecular weight of pediocin during the amino acids series was 4624 or 4628 Dalton depending on the presence or absence of disulfide bonds. The molecular weight of pediocin was estimated by SDS-polyacrylamide gel electrophoresis and it was found 6094.8 Dalton (Niamah, 2010), while it was 16500 Dalton by gel filtration method (Gonzalez and Kunka, 1987). Electrospray mass spectrometry showed disulfide bonds in pediocin structure which is oxidizing molecules causing an increase in the molecular weight of pediocin (Daba *et al*.,1994).

Action mode of pediocin

The cytoplasmic membrane of bacteria such as lactic acid bacteria is closely related genetically and it is the target of pediocins. Pediocin has three mechanisms to inhibit the bacteria: -1) work pores made in cytoplasm membrane of cell target, 2) collapse pH intracellular of bacteria target, 3) inhibition of proton motive force for energy production in the bacterial cell. Pediocin was linked with cell target through active sites such as negative charges of phospholipids groups in cell wall. Past studies which related between amount lipoteichoic acid in cell wall of gram positive bacteria (G^+) and increased pediocin activity against (G^+) bacteria. The higher pediocin activity when acid concentration is increased in the walls of bacterial cells (Perez *et al*., 2015; Wang *et al*., 2015).

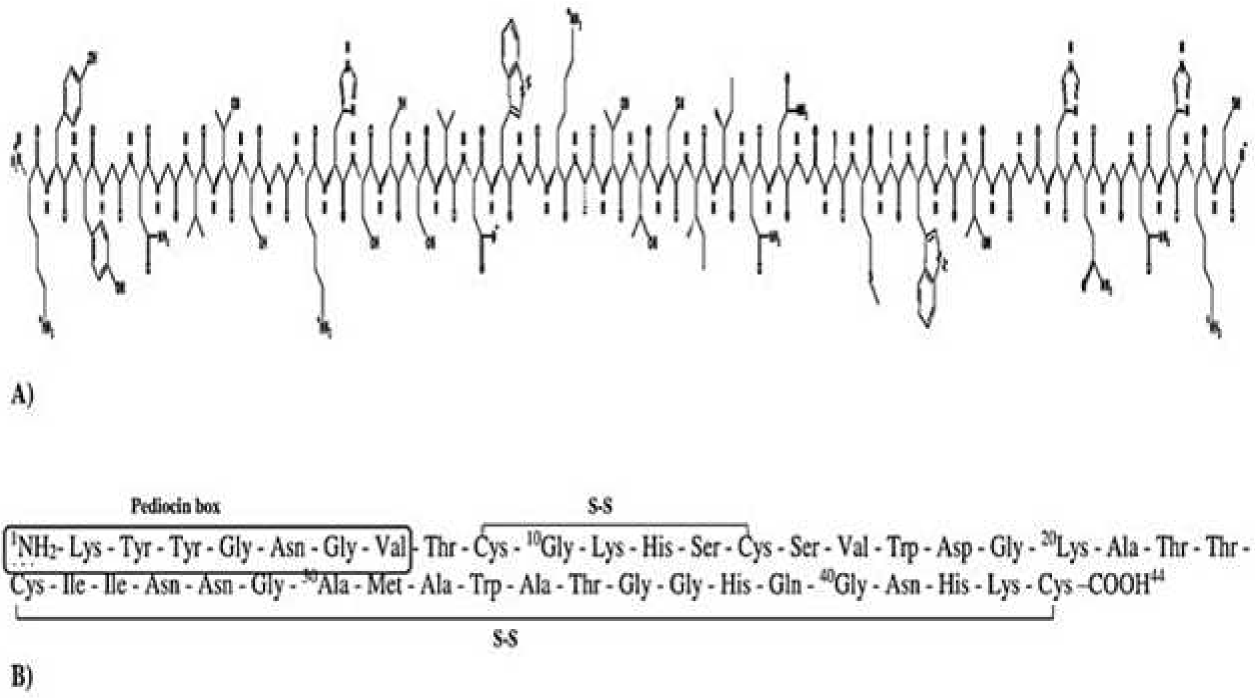

Fig. (1): Pediocin structure. A) amino acid structure of pediocin, B) active sites of pediocin

Montville and Chen (1998) showed three main steps for the inhibition activity of pediocin against bacteria 1) linking pediocin with cytoplasm membrane of the targeted cell. 2) entering pediocin molecule into the targeted cell. 3) formation of complexes between pediocin and the cell components such as enzymes, proteins and DNA. Then autolysis of the targeted cell (Fig 2). However, *Bacillus* and *Clostridium* spores do not adsorb the pediocin. After their germination and growth in culture media, the sensitive bacteria cells adsorb the pediocin and are inhibited (Todorov *et al*., 2016).

Properties of pediocin

The Class II of bacteriocins is divided into the pediocin-like bacteriocins and the two-peptide bacteriocins. The known produced pediocin from species of *Pediococcus* genus is mostly small structure, hydrophobic peptides, thermally stable and bears low temperatures even at -80°C. Pediocin activity is maintained in a wide range of pH at 2-10. It is sensitive to general proteases enzymes such as pepsin, papain and trypsin (Table 1).

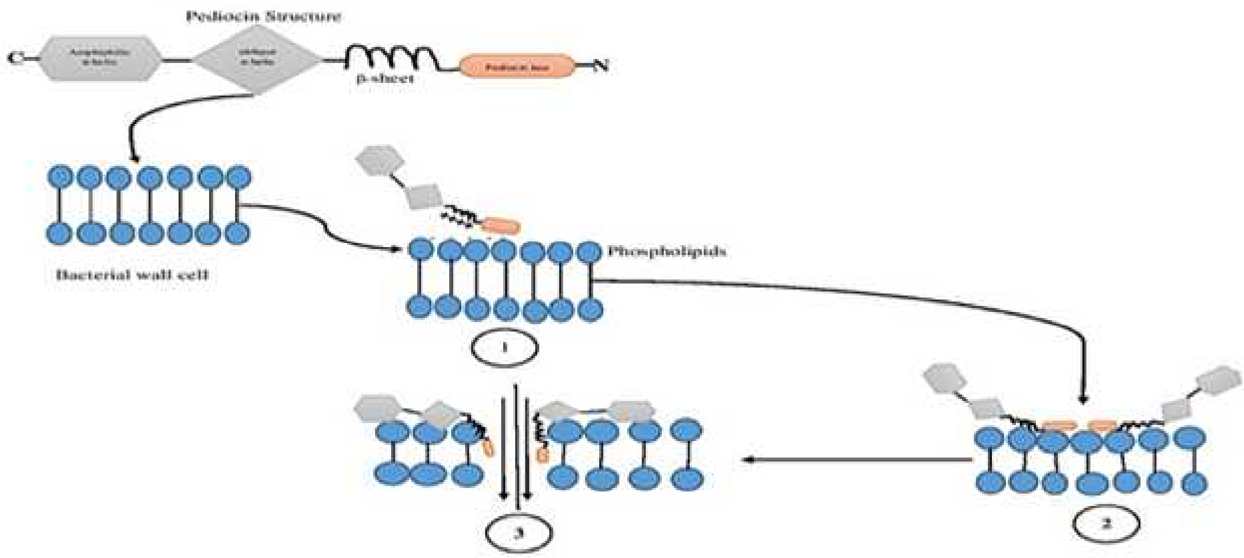

Fig. (2): The schematic diagram of the pores formation of pediocin in sensitive cell walls, (1) Adsorption pediocin on the cell walls, (2) hydrophobic interaction, (3) pores formation in cell wall.

Table (1): The properties of some pediocin types (heat stable, pH and proteases enzymes resistance).

[+: Activity reaction (50-100) %, L: Low activity (<50), ND: not tested].

	Treatments								
Pediocin types	**Temperatures**		**pH**	**proteases enzymes**					**References**
	100°C/ 30 min	121°C/ 15min	2-10	Trypsin	chymotrypsin	Pepsin	Proteinase K	Papain	
AcH	ND	+	+	L	L	ND	L	L	Bhuni *et al.* (1998)
F, A	ND	+	+	L	L	ND	L	L	Osmanagaoglu *et al.* (1998)
ACCEL	+	+	+	ND	ND	ND	ND	ND	WU *et al.* (2004)
SA-1	+	+	+	+	+	+	L	+	Anastasiadou *et al.* (2008)
AK	+	+	+	±	ND	ND	L	L	Niamah (2010)
PA-1	+	+	+	L	L	ND	L	ND	Osmanagaoglu *et al.* (2011)
NCIM	+	+	+	L	L	L	L	L	Mandal *et al.* (2014)

Spectrum of pediocin activity

Generally, the pediocin has inhibition activity against gram positive bacteria and gram negative bacteria (Table 2). Daeschel and Klaenhammer (1985) studied the inhibitory spectrum of pediocin products from *P. pentosaceus* FBB61 and *P. pentosaceus* L7230 which showed inhibition activity against *Pediococcus* spp., *Lactobacillus brevis* and *Lactobacillus plantarum*. The starters contain *P. acidilactici* had higher inhibition activity against *Listeria monocytogenes*. These starters are used in fermented meat and other food products (Lewus *et al*., 1991).

Green *et al*. (1997) and Anastasiadou *et al*. (2008) showed spectrum of pediocin SA-1which is produced from *P. acidilactici* NRRL B 5627 with inhibition activity against bacteria that spoilage food or pathogenic bacteria such as *Bacillus cereus* LMG13569, *Clostridium sporogenes* NCTC533, *Clostridium thiaminolyticum* ATCC15579, *Enterococcus faecalis* NCTC8176, *Leuconostoc mesenteroides* ATCC19254, *Micrococcus flavus* ATCC400, *Listeria monocytogenes* ATCC19111, *Salmonella carnosus* LMG13564 and *Lactobacillus brevis* ATCC8287. But it did not inhibiters activity against *Salmonella enteritidis* ATCC13076.

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Table (2): Some pediocin types produced by *Pediococcus* genus and indicator bacteria.

Pediocin type	Produced bacteria	Indicator bacteria	References
AcH	*P. acidilactici* H	*Lactobacillus plantarum* *Lactobacillus* spp.	Biswas *et al*. (1991)
SJ-1	*P. acidilactici* SJ-1	*Clostridium perfringens* *Listeria monocytogenes*.	Schved *et al*. (1993)
L50	*P. acidilactici*	*L. monocytogenes*	Cintas *et al*. (1995)
AcM	*P. acidilactici* M	*Staphylococcus aureus* *L. monocytogenes* *C. perfringens,* *Bacillus coagulans* *B. cereus* *Aeromonas hydrophila.*	Elegado *et al*. (1997)
ST18	*P. pentosaceus* ST18	*Pediococcus* spp. *Listeria innocua* *Lactobacillus plantarum*.	Todorov and Dicks (2005)
PD-1	*P. damnosus*	*Listeria* spp.	Anastasiadou *et al*. (2008)
AK	*P. acidilactici* AK	*S. aureus*	Niamah (2010)
NV 5	*P. acidilactici* LAB 5	*Enterococcus faecalis* *Leuconostoc mesenteroides*	Mandal *et al*. (2010)
PA-1	*P. acidilactici* J347-29	*Oenococcus oeni*	Díez *et al*. (2012)
P34	*P. pentosaceus* 34	*Listeria monocytogenes* *Enterococcus faecium* *E. faecium* *E. faecalis*	Kaur *et al*. (2014)
KJBC11	*P. pentosaceus* KJBC11	*Listeria monocytogenes*	Sadishkumar and Jeevaratnam (2017)

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Pediocin applications in foods

During in the last decade of the 21th century these was great concern about its side effect of chemical additives in food which arises the emergence of many diseases it is believed that the chemical additives are the direct or indirect causes of these diseases. Researchers worldwide were looking for natural food or fresh food with no additives, or discover natural substances that are highly effective in food preservation, including bacteriocins such as Nisin or Pediocin which are used to improve food safety and increase its shelf life without side effect. Pediocin has been commercially produced under the name "Alta ™ 2341" and is used in the USA and European countries as a preservative especially in meat products to inhibit the growth of *Listeria monocytogenes* (Porto *et al*., 2017).

Microbial starters (bacteria and yeast) are capable to produce inhibitory proteins were used in inhibition of pathogenic bacteria. These starters are traditionally used in manufacturing of fermented sausage, fermented vegetables, fermented olives, dairy products and fermented grains food (Niamah, 2017; Niamah *et al*., 2017a). There are four mechanisms for adding pediocin to food, 1) Using Pediocin producing bacteria as starter in fermented food. 2) Adding purified or semi-purified pediocin into food matrix. 3) Using fermented food by pediococci and introducing as a component in the manufactured food. 4) Food packaging films are incorporated with pediocin.

In meat and meat products

Powdered Pediocin was produced from milk-based media and applied to plastic bags for meats and poultry packaging samples. The samples were inoculated with *Listeria monocytogenes* and storage for 12 weeks at 4°C. The bags reduced the viability numbers of *L. monocytogenes* after this storage time (Ming *et al*., 1997). Nieto-Lozano *et al*. (2010) showed that the highest activity of pediocin PA-1 (5000 bacteriocin units/ml) produced by *Pediococcus acidilactici* MCH14 against *L. monocytogenes* and *Clostridium perfringens* in Spanish dry-fermented sausages samples. The pediocin reduced the numbers of this bacteria after storage time for 60 days at 10°C. *P. acidilactici* LAB 5 which produce pediocin was isolated from vacuum-packed fermented meat product. This pediocin has higher inhibition active against food spoilage and human pathogenic bacteria like some species of *Enterococcus*, *Listeria*, *Leuconostoc* and *Staphylococcus* (Mandal *et al*., 2008).

The pediocin AcH/PA-1 produced by *Pediococcus pentosaceus* OZF isolated from human breast milk and applied on chicken meat products which were radiated and inoculated with Log. 5.0 CFU/g of *L. monocytogenes* and purified pediocin. A significant decrease of *L. monocytogenes* viability after packaging and store of 4 weeks at 4°C (Kiran and Osmanagaoglu, 2014). In another study, the pediocin produced by *P. pentosaceus* and *Murraya koenigii berries* used as the antimicrobial and antioxidant in goat meat emulsion during storage period of 9 days at 4 °C. The effects of pediocin and *Murraya koenigii berries* combination had higher inhibition activity against *L. innocua* after storage period (Kumar *et al.*, 2017).

In milk and dairy products

Milk and dairy products are the best media for pathogenic bacteria and bacteria related with food spoilage bacteria such as *Listeria monocytogenes, Salmonella* sp., *Staphylococcus aureus*, *Campylobacter* sp. and *Yersinia* sp. (Niamah, 2012; Abbas *et al.*, 2017). Motlagh *et al.* (1992) mentioned pediocin activity against *L. monocytogenes* in cottage cheese, sauce cheese and ice cream after storage at 4°C and 32°C. The numbers of *L. monocytogenes* bacteria reduced in cheese samples with pediocin during storage period compared with the control sample. Direct application of pediocin PA-1 produced by *Pediococcus* genus as an antibacterial against *L. monocytogenes*. *Pediococcus* genus is not able to ferment lactose quickly, making it metabolically unsuitable for dairy fermentation products. In spite of growth of *Pediococcus* species being grow weakly in milk and dairy products, but using Log. 9.0 CFU/mL of *Pediococcus* as starter inhibited the growth of *L. monocytogenes* in milk samples (Ming *et al.*, 1997). The produced Pediocin PA-1 by *Pediococcus pentosaceous* NCDC 273 when it used 2.04×10^5 AU/mL of pediocin PA-1 increased the shelf life of raw buffalo milk and reduced the total viable numbers of bacteria (Verma *et al.*, 2017).

In other foods

Despite the prolongation of shelf life in food products by the addition of organic acids such as sorbic acid, benzoic acid and lactic acid, interest about the use of these organic acids have led investigators to search, produced bacteriocins from lactic acid bacteria is using for food preservation. Pediocin has a distinct scientific application to inhibit *L. monocytogenes* in the production of some fermented foods, especially in planned fermentation process where specific bacteria of starter cultures are used. By mixing pediocin through the formulation of the raw product, infection and pollution bacteria problems in the final product could be reduced. Muriana (1996) who showed to add pediocin to eggs whole led to reduce the survival of *L. monocytogenes*. Edible film coatings added with produced pediocin from *Pediococcus pentosaceus* could also be a great alternative to preserve minimally processed papaya (*Carica papaya* L.) fruits (Narsaiah *et al.*, 2015).

Conclusions

Pediocins produced by *Pediococcus* spp. have promising antimicrobial activity. It is possibly used as direct to safe antimicrobial food addition on could by produced by the added starters bacteria. Presently, partly purified or concentrated of produced pediocin used as a food additive a substitute for preservatives of foods. Addition of pediocin pour concentrated beside started may be more affective and could be promising for new functional food production. Pediocin can use within functional food. Producing mixture of bacteriocins by lactic acid bacteria with high effectiveness and low cost could certainly improve their opportunities to use in the consumer foods.

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