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Characterization of Pediocin PaF-11 from *Pediococcus acidilactici* F-11 as Biopreservatives

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Pediocin PaF-11 is bacteriocin from Pediococcus acidilactici F-11. The aim of this study was to know the characteristics of pediocin PaF-11 as food biopreservatives. This study used P. acidilactici F-11 and Lactobacillus pentosus LB 42 as the pediocin producing and indicator strain, respectively. Both strains were obtained from the Food Nutrition Culture Collection, Gadjah Mada University. The P. acidilactici F-11 was grown to produce the pediocin in TGE liquid medium at a pH of 6.5 and an incubation temperature of 37°C for 18 hours. The pediocin PaF-11 was extracted and purified according to the adsorption-desorption methods and its activity was determined by the well-inhibition methods. The stability of pediocin against temperature and storage condition was characterized. The purified pediocin PaF-11 was stable after heating at a temperature of 100°C for 30 minutes or temperature 121° C for 15 minutes and storage at a temperature of 30oC and 4oC for 11 and 13 weeks. It was also active over a wide range of pH values from 3 to 8. The stability of pediocin PAF-11 to temperature is also supported by the following amino acid sequence results. Amino acid from the N-terminal of pediocin PAF-11 was sequenced: MKKIEKLTEKEMANIIGGKYYGNGVTCGKHSCSVDWGKATTCIINNGAMAWATGGHQGNHKC). The pediocin PaF-11 is in the consensus sequence YYGNGVXCXXXXCXVXXXXA which indicates that pediocin PaF-11 is belong to class IIa bacteriocin. These characteristics make pediocin PaF-11 suitable as a food preservative, especially products that involve processing on pH such as in the process of fermentation and processing at high temperatures as well as storage.

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Introduction

One of the antibacterial substances that is suitable for food preservation is bacteriocin produced by lactic acid bacteria. Bacteriocin is a peptide or peptide complexes are synthesized in ribosomes. nisin produced by Lactococcus lactis subsp. lactis is the only commercial product that has a status of GRAS (Generally Recognized as Safe) and accepted by the USA FDA (Food and Drug Administration) as a food preservative. Nisin has been applied in most of the major food-producing country. However, nisin are less effective against gram-negative bacteria, yeast and fungi and solubility as well as its stability decreased significantly on the conditions of the meat which is neutral and alkaline.

Pediocin of Pedioccocus draws attention to researched because some researchers have proved that the pediocin of P. acidilactici, P. damnosus, P. parvulus and P. pentosaceus has the ability to inhibit pathogens and spoilage bacteria so that it could potentially be used as a food preservative. The potential is supported by the benefits that can improve the weaknesses of nisin, among others in its stability in a wide range of pH (Huang et al., 2009; Anastasiadou et al., 2008a; Todorov and Dicks, 2009; Osmanagaoglu, et al., 2011) as well as heat treatment (Anastasiadou, et al., 2008b).

On the assumption that a specific bacteriocin will have its own unique properties and usefulness in targeting microbial pathogens, isolation and purification of new bacteriocins will always prove beneficial. In-depth characterization of the purified form of new isolates will provide information for economically viable mass production and downstream processing (Coventry et al., 1996), together with useful data for making manipulations to improve bacteriocin properties and effectiveness (De Vuyst and Vandamme, 1994), and to hasten consumer acceptability of bacteriocintreated products.

The pediocin PaF-11 produced by Pediococcus acidilactici F-11 has potential use as biological food preservatives. In order to use it in the most effective way as biological preservative, the most important factors to be considered are stability at various pH, temperature and storage condition of pediocin PaF-11. To support the application of pediocin PaF-11, research that aims to know sequence of amino acids and the molecular weight were also needed.

Materials and Methods

Bacterial cultures and media

P. acidilactici F-11 and *Lactobacillus pentosus* LB42 were used as pediocin PaF-11 producer and indicator bacteria respectivelly, both of which were obtained from the Food and Nutrition Culture Collection (FNCC), Center for Food and Nutrition Studies of Gadjah Mada University in Yogyakarta. *P. acidilactici* F-11 produced pediocin PaF-11 in the TGE broth consist of tryptone (1%), glucose (1%), yeast extract (1%), tween 80 (0.2%), $MnSO_4.H_2O$ (0,005%), and $MgSO_4.7H_2O$ (0,0056%) at pH 6.5 within 18 hours incubation at 37 °C. The assay plate had a bottom layer of TGE agar (TGE broth plus 1.5% agar) and a top layer of TGE soft agar (TGE broth plus 0.75% agar)(Biswas et al., 1991).

Production and purification of pediocin PaF-11

Partial purification of the pediocin PaF-11 was performed by the adsoprtion desorption methods (Yang et al., 1992) was applied. Fresh culture of P. acidilactici F11 was inoculated at a rate of 0.1% in modified TGE broth at pH 6.5 and pre-sterilized at 15 psi for I5 min (Biswas et al., 1991). P. acidilactici F11 was grown 18 hour in TGE broth. Broth culture was heated at 100°C for 30 minutes to kill the cells after its pH adjusted to 6.5. The mixture was kept at 4 °C for 24 hour by mixing with stirrer. The cells were harvested by centrifugation at 15,000 rpm for 15 minutes. After the cells had been washed with 5mM sodium phosphate (pH 6.5), the cells were then resuspended in H2O, and 0.1 M NaCI was added and the pH of the mixture was adjusted to 2,0. The mixture was kept at 4°C for 24 hr by mixing with stirrer. Cell suspensions were then centrifuged at 29,000 rpm for 20 minutes, and the supernatants were freeze-dried.

Antimicrobial activity assay of pediocin PaF-11

Pediocin PaF-11 activity was determined by well diffusion agar methods. The assay plate had a bottom layer of TGE agar (TGE broth plus 1.5% agar) and a top layer of TGE soft agar (TGE broth plus 0.75% agar) and was seeded with about 10^6 *L. pentosus* LB42 cells. Then, 5mm wells were bored out and each was filled with 20µl of the pediocin PaF-11 fractions which were serially diluted. The agar plates were incubated at 37°C overnight. The highest dilution that produced a clear zone was multiplied by 50 (1ml/20µl) to obtain the activity units per milliliter (Biswas et al., 1991; Kim et al., 1993).

Effect of heat treatment on antimicrobial activity of pediocin PaF-11

Analysisi of the effect of heat treatment on antimicrobial activity of pediocin PaF-11 was done by heating the pediocin PaF-11 at 100°C for 15 and 30 minutes (Huang et al., 2009) and autoclaving at 121°C for 5 and 15 minutes (Schneider et al., 2006; Huang et al., 2009). All samples were cooled and assayed for activity (Elegado et al., 1997; Osmanagaoglu et al., 1998).

Effect of pH on antimicrobial activity of pediocin PaF-11

Pediocin PaF-11was adjusted with sterile 10 mM/l NaOH or 10 mM/l HCI to different pH levels between 3 (Osmanagaoglu et al., 2001) to 10. Samples were maintained at 37 °C for 2 hours (Huang et al., 2009). The samples were then adjusted to pH 7.0 with sterile 4 mM/l phosphate buffer and assayed for activity **(**Elegado et al., 1997; Osmanagaoglu et al., 1998).

Effect of storage condition on antimicrobial activity of pediocin PaF-11

For storage stability determination, pediocin was storage in the cool room at 4°C and room with temperature 25-30°C (Wu et al., 2004; Mathys et al., 2007; Anastasiadou, et al., 2008a). Both sampels were assayed for activity every week periodically.

Amino acid sequencing of pediocin PaF-11

Plasmid isolation of P. acidilactici F-11 with alkaline lysis done by Sambrook et al., (1989) and Kalmokoff et al., (2003) methods. Plasmid DNA of P. acidilactici F-11 isolated then used as templates for the gene pediocin (papA) amplification through the PCR analysis (Huang et al., 2009; Todorov and Dick, 2009). The Primer is designed based on the sequence of papA gene in the GenBank database uses the program primer3 online (http://frodo.wi.mit.edu/primer3/), there were: primer 1 (Forward) 5'-GCGCGTATTAAGGATAATTT-3' and primer 2 (Reverse) : 5'-TTTATTGATGCCAGCTCAGC-3'.
Nucleotide sequencing of DNA is done by DNA sequenser. Molecular weight pediocin PaF-11 performed by calculation used to use a program that refers to the EnCor Biotechnology Inc. © 2011 Analysis.

Results and Discussion

Stability of pediocin PaF-11 on heat treatment

Heat treatment at 100 °C for 15 and 30 minutes and autoclaving at 121 °C for 5 and 15 minutes did not destroy the activity of pediocin PaF-11 activity. Antibacterial activity of pediocin PaF-11 after heating at 100°C and autoclaving at 121°C using *L. pentosus* LB42 as indicator seen in Table 1.

Table 1. Antibacterial activity of pediocin PaF-11 after heat treatmen using *L. pentosus* LB42 as indicator

Heat treatment	Pediocin PaF-11 activity (AU/ml)
100 °C for 15 min	1500
100 °C for 30 min	1500
121 °C for 5 min	1500
121 °C for 15 min	1500

Previous research showed that pediocin PaF-11 resistant to heat. It have been reported that bacteriocin from *P. acidilactici strain Hansen* remain stable after heating at 115°C for 15 min (Lozano et al., 2002) *P. acidilactici* SJ-1 at 65-121°C (Schved et al ., 1993), *P. acidilactici M; P. acidilactici F*; *P. acidilactici* C20; and *P. acidilactici* PO2 at 121 °C for 15 min (Elegado et al., 1997; Osmanaglaogu et al ., 1998; Halami et al., 2000; and Coventry et al., 1995). The recently isolated and characterized pediocin SA-1 from *P. acidilactici* NRRL B5627 was also found to be heat stable for up to 60 min at 121°C **(**Papagianni and Anastasiadou, 2009).

The thermotolerance feature of pediocin PaF-11 might be related to the molecular structure of the bacteriocin, usually composed by small peptides without tertiary structure (Parada et al., 2007) and has low molecular weight (Osmanagaoglu et al., 2001; Papagianni dan Anastasiadou, 2009). Another posibility is that because pediocin PaF-11 contain cysteine (Nugroho and Rahayu, 2003). These characteristics make pediocin PaF-11 suitable as a food preservative, especially for developing products that involve process at high temperature such as pasteurization.

Stability of pediocin PaF-11 on pH treatment

Pediocin PaF-11 was found to be stable over a wide pH range between 3 and 8 (Table 2). Activity was lost at pH 9, the loss of activity at higher pH could be due to degradation of the molecule (Osmanaglaogu et al., 1998). This is similar to the properties reported for bacteriocins produced by other lactic acid bacteria such as bacteriocin from *P. acidilactici* SJ-1 and *P. acidilactici F* stabil at pH 3-9 (Schved et al.,1993; Osmanaglaogu et al.,1998); *P. acidilactici M* stabil at pH 1-12 (Elegado et al.,1997), *P. acidilactici* NRRL B5627 stabil at pH 3-12 (Anastasiadou et al., 2008), *P. pentosaceus* ST18 stabil at pH 2-12 (Todorov dan Dicks, 2005) *P. pentosaceus 05-10* stabil at pH 2-8 (Huang et al ., 2009) dan *P. pentosaceus* Pep1 stabil at pH 3-8 (Osmanaglaogu et al ., 2001).

Table 2. Antibacterial activity of pediocin PaF-11 after pH treatment using L. pentosus LB42 as indicator

pH	Pediocin PaF-11 activity (AU/ml)
3	1500
4	1500
5	1500
6	1500
7	1500
8	1200
9	0
10	0

The increased antibacterial activity observed at low pH may be the result of aggregation of hydrophilic peptides is less likely to occur, and, thus, more molecules should be available to interact with sensitive cells (Jack et al., 2005). Stability of pediocin PaF-11 at a wide range of pH (between 3 and 8) make it suitable as a food preservative, especially for developing products that involve process wherein pH plays important role such as fermentation

Stability of pediocin PaF-11 on storage treatment

Activity of pediocin PaF-11 remained unaffected after 11 weeks storage at 4°C, and decreased after 11 weeks. Total loss of bacteriocin activity was already observed after 11 weeks storage at 30°C (Table 3). The loss of activity of pediocin during storage in the presence of air could be due to oxidation of methionine. In liqiud preparation, aggregation of the molecules can also produce an apparent loss (Naidu, 2000).

Similar result, pediocin SA-1 from *P. acidilactici* NRRL B5627 was also found to be stable during storage. Storage for 4 weeks at -80, -20, 4 and 30°C did not affect its antimicrobial activity. This was not impaired even following incubation at 30°C for 1 week at pH values ranging between 3.0 to 12.0 **(**Papagianni and Anastasiadou, 2009**).** Activity of bacteriosin from *P acidilactici* UVA1 unaffected after one month storage at 4°C at pH values from 2 to 8. At pH 10 and 11, total loss of activity was already observed after 1 week storage and only 60% of the initial activity was still measurable after 1 month at pH 9 **(**Mathys et al., 2007).

Table 3. Antibacterial activity (b) of pediocin PaF-11 after storage at 4 °C and 30 °C using *L. pentosus* LB42 as indicator

Storage time (Week)	Pediocin PaF11 activity (AU/ml)	
	Storage temperature (4°C)	Storage temperature (30°C)
1	1500	1500
2	1500	1500
3	1500	1500
4	1500	1500
5	1500	1250
6	1500	1500
7	1500	1500
8	1500	1500
9	1500	500
10	1500	500
11	1500	250
12	1500	0
13	1000	0

Amino acid sequence of pediocin PaF-11

The amino acid from the N-terminal of pediocin PAF-11 was sequenced : Methionine-Lysine-Lysine-Isoleucine-Glutamate-Lysine-Leusine-Threonine-Glutamate-Lysine-Glutamate-Methionine-Alanine-Asparagine-Isoleucine-Isoleucine-Glycine-Glycine-Lysine-Tyrosine-Tyrosine-Glycine-Asparagine-Glysine-Valine-Threonine-Cysteine-Glysine-Lysine-Histidine-Serine-Cysteine-Serine-Valine-Aspartate-Tryptophan-Glycine-Lysine-Alanine-Threonine-Threonine-Cysteine-Isoleucine-Isoleucine-Asparagine-Asparagine-Glysine-Alanine-Methionine-Alanine-Tryptophan-Alanine-Threonine-Glycine-Glycine-Histidine-Glutamine-Glycine-Asparagine-Histidine-Lysine-Cysteine

(**MKKIEKLTEKEMANIIGGKYYGNGVTCGKHSCSVDWG KATTCIINNGAMAWATGGHQGNHKC).** *Based on pediocin PaF-11 sequence ie**MKKIEKLTEKEMANIIGGKYYGNGVTCGKHSCSVDWG KATTCIINNGAMAWATGGHQGNHKC**,* and calculation using a program that refers to the EnCor Biotechnology Inc. © 2011 Analysis, the pediocin PaF-11 has a molecular weight of around 6.65. kDa.

Table 4. Nuclotide sequence of encoding gen and amino acid sequence of pediocin PaF-11

DNA	ATG	AAA	AAA	ATT	GAA	AAA	TTA	ACT	GAA	AAA	GAA	ATG	GCC	AAT	ATC	ATT
Amino Acid	Met	Lys	Lys	Ile	Glu	Lys	Leu	Thr	Glu	Lys	Glu	Met	Ala	Asn	Ile	Ile
Symbol	M	K	K	I	E	K	L	T	E	K	E	M	A	N	I	I
DNA	GGT	GGT	AAA	TAC	TAC	GGT	AAT	GGG	GTT	ACT	TGT	GGC	AAA	CAT	TCC	TGC
Amino Acid	Gly	Gly	Lys	Tyr	Tyr	Gly	Asn	Gly	Val	Thr	Cys	Gly	Lys	His	Ser	Cys
Symbol	G	G	K	Y	Y	G	N	G	V	T	C	G	K	H	S	C
DNA	TCT	GTT	CAC	TGG	GGT	AAG	GCT	ACC	ACT	TGC	ATA	ATC	AAT	AAT	GGA	GCT
Amino Acid	Ser	Val	Asp	Trp	Gly	Lys	Ala	Thr	Thr	Cys	Iso	Iso	Asn	Asn	Gly	Ala
Symbol	S	V	D	W	G	K	A	T	T	C	I	I	N	N	G	A
DNA	ATG	GCA	TGG	GCT	ACT	GGT	GGA	CAT	CAA	GGT	AAT	CAT	AAA	TGC		
Amino Acid	Met	Ala	Trp	Ala	Thr	Gly	Gly	His	Gln	Gly	Asn	His	Lys	Cys		
Symbol	M	A	W	A	T	G	G	H	Q	G	N	H	K	C		

Remarks : Class II a bacteriocin concensus : ***KYYGNGVXCXXXXCXVXXXXA***
Hidrofobic amino acid : metionin, isoleusin, leusin, alanin, tirosin, valin, dan triptofan

The pediocin PaF-11 is in the consensus sequence **YYGNGVXCXXXXCXVXXXXA** which indicates that pediocin PaF-11 is belong to class IIa bacteriocin. The sequence of pediocin PaF-11 has a high homology (100%) with pediocin PA-1 (from *P. acidilactici* PAC 1.0 (Marugg et al., 1992) and *P. pentosaceus* IE-3); prepediocin PA-1 (from *P. acidilactici* K10) (Moon dan Kim, 2004)**,**; pediocin AcH (from *P. acidilactici* H Motlagh, et al., 1992; *P. acidilactici* LB42-923 (Motlagh et al., 1994); *P. parvulus* ATO77 (Miller et al., 2005),and *P. pentosaceus* S34 Miller et al., 2005) and prepediocin CP2 from *P. acidilactici* MTCC 5101 (Balgir et al., 2010), and pediocin from *P. acidilactici* CFR K7 (Halami et al 2005) and *P. acidilactici* PED 01 (Laoye and Dodd, 2008).

Based on pediocin PaF-11 sequence and calculation using a program that refers to the EnCor Biotechnology Inc. © 2011 Analysis, the pediocin PaF-11 has a molecular weight of around 6.65. kDa.That result showed that pediocin PaF-11 has low molecular weight, is similar to pediocins isolated by various *Pediococcus,* ie the pediocin AcH (from *P. acidilactici* PAC 1.0; *P. acidilactici* LB42-923; *P. parvulus* ATO77) and pediocin PA-1 (from *P. acidilactici* K10; P. pentosaceus IE-3; *P. acidilactici* MTCC 5101) whith molecular weight around 6644 Da. The pediocin PaF-11 is a larger molecule compared to the 3660, 3500, 5370, 5000. 6500, dan 4623 Da molecule of of pediocin SA-1, PA-1, SM-1, K23-2, 05-10, and AcH.

These pediocin PaF-11 characteristics make pediocin PaF-11 suitable as a food preservative, especially products that involve processing at high temperatures because protein with low molecular weight has stability to heat process. The molecular weight of pediocin PaF-11 showed that it resembles known pediocins and belongs therefore to the class IIa of bacteriocins by according to the classification Drider et al., (2006); Aly et al., *(*2006) dan Nissen-Meyer dan Nes (1997).

Conclusions

Pediocin PaF-11 was stable at pH of 3-8 and autoclaving (121°C for 15 min). Pediocin PaF-11 was stable during 11 and 13 weeks storage at 30°C and 4°C respectively. The pediocin PaF-11 is in the consensus sequence **YYGNGVXCXXXXCXVXXXXA** which indicates that pediocin PaF-11 is belong to class IIa bacteriocin. Based on that sequence, the pediocin PaF-11 has a molecular weight of around 6.65. kDa. These pediocin PaF-11 characteristics make pediocin PaF-11 suitable as a food preservative, especially products that involve processing at high temperatures because protein with low molecular weight has stability to heat process.

Conflict of Interest

All the authors declare that they have no conflict of interest.

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