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Characterization of Bacteriocin Produced by *Pediococcus acidilactici* Isolated from Fermented Pork in Thailand

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ABSTRACT

The bacteriocin produced by *Pediococcus acidilactici* isolated from fermented pork (nham) was partial purified by means of ammonium sulphate fractionation. Thebacteriocin was exhibited antimicrobial activity against variety of food spoilage and pathogenic bacteria. It was shown to be heat tolerant and stable over a wide pH range, and apparently most stablein the lower part of that range. The proteolytic enzyme, trypsin completely inhibit the activity of bacteriocin.

Key words : bacteriocin, characterization, *Pediococcus acidilactici* fermented pork

INTRODUCTION

Lactic acid bacteria produce a variety of antibacterial compounds such as organic acids, diacetyl, hydrogen peroxide, reuterin and bacteriocins. Among these, bacteriocins have gained increasing interest. Bacteriocins are proteinaceous compoundsthat have bactericidal activity against a limit range of microorganisms (Klaenhammer, 1988; Juven *et al.*,1992). On the other hand, bacteriocins produced by *Pediococcus* spp. have been reported to exhibit broader spectra of antimicrobial activity (Daeschel and Klaenhammer, 1985; Bhunia *et al.*,1988).

In this study, the bacteriocin produced by *Pediococcus acidilactici* isolated from "nham" a traditional fermented pork of Thailandwas partially purified and characterized, in order to use as a natural and safe preservative for processed foods.

MATERIALS AND METHODS

Bacterial strains and media

The bacteriocin-producing strain, *P. acidilactici* was isolated from nham. It was identified as *P. acidilactici* by Gram staining, catalase test, cell morphology, growth at selected temperatures, thermal resistance, and carbohydrate fermentation pattern (Weiss,1992). All lactic acid bacteria usedin this study were grown in MRS broth at 30°C for 18 h, and the non-lactic acid bacteria in Tryptic Soy broth at 30°C for 18 h. *Clostridium perfringens* was cultured in Thioglycolate agar under anaerobic condition.

Production of bacteriocin from *P. acidilactici*

P. *acidilactici* was grown in 1l of MRS broth at 30°C for 18h without shaking. Cells were removed by centrifugation (12,000 rpm for 10 min,4°c) and thesupernatant fluid was neutralizedto pH 5.0. This preparation is designated crude bacteriocin.

Bacteriocin assay

Each bacteriocin preparation was serially diluted twofold dilution, and 5 l samples were spotted on freshly prepared lawn of the indicator strain. Bacteriocin activity was measured by spot-on-lawn assay (Mayr-Harting *et al.*,1972) using *Lactobacillus plantarum* as an indicator strain.One arbitary unit (AU) was defined as 5 l of the highest dilution of the bacteriocin solution causing a definite zone of inhibition on the lawn of the indicator organism.

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Ammonium sulphate precipitation

Solid ammonium sulphate was added to the crude bacteriocin (1l) and brought the saturation to 60% by stirring, and then centrifuge at 18,000 rpm for 30 min. The precipitate was dissolved in 100 mM Sodium phosphate buffer (pH 6.5) and extensively dialysed against the same buffer. The dialysate, containing bacteriocin activity was stored at -20°C until used. This preparation is called partially purified bacteriocin.

Inhibitory spectrums of the antimicrobial substance against foodborne pathogens

Nine pathogenic organisms including seven Gram positive bacteria, and two Gram negative bacteria were tested for their sensitivity by using the spot-on-lawn assay. The 5 µl of partially purified bacteriocin was assayed on lawn of pathogenic strain.

Bactericidal action

Log-phase cells of *L. plantarum* (indicator strain) were prepared by incubation at 30°C for 6h and were incubated with the bacteriocin preparations, obtained by precipitation with ammonium sulphate, at 30°C for another 6h. At appropriate intervals, the viable indicator cells were counted on MRS agar plates.

Effect of Trypsin and detergents on bacteriocin

Sensitivity of the bacteriocin to trypsin and detergents was measured. Samples of partially purified bacteriocin were exposed to trypsin (Sigma Chemical Co.) at a final concentration of 0.5 mg/ml. Triton x-100, Tween 80, and SDS were also used to study sensitivity of bacteriocin to detergents. Reactions were carried out at 30°c. After incubation for 6h, 5 µl volumes were withdrawn from each reaction mixture and tested for activity against the indicator strain.

Stability at various pH values

The pH of partially purified bacteriocin was adjusted with NaOH or HCl to the following levels : 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, and 10.0. After storage for 6h at 30°c, the samples were assayed for activity with the indicator strain.

Effect of heat-treatment on bacteriocin

To test the heat-stability, the partially purified bacteriocin were heated at 60°c, 80°c, 100°C for 60 min, 60 min, and 30 min, respectively or autoclaved at 121°C for 15 min, and assayed for inhibitory activity against the indicator strain.

RESULTS AND DISCUSSION

Inhibitory spectrum of the bacteriocin

The inhibitory spectrum is shown in Table 1. The bacteriocin activity was demonstrated against various foodborne pathogens including *Listeria monocytogenes, Staphylococcus aureus, Bacillus cereus* and *Clostridium perfringens*. However, tested Gram negative bacteria namely *Salmonella enteritidis* and *Salmonella derby* were not antagonized by bacteriocin.

One characteristic of classical bacteriocins is a narrow spectrum of activity (Tagg *et al.*,1976). Well-defined bacteriocins produced by lactobacilli usually have inhibitory activities restricted to closely-related species (Juven *et al.*, 1992). The broad spectrum of activity of the bacteriocin produced by *P. acidilactici* isolated from nham, will be an excellent candidate as food biopreservative in extending shelf-life of foods.

Among the foodborne pathogens, inhibition was more pronounced in *L. monocytogenes* than in the others (inhibitionzone 23 mm in diameter, data not shown). The presence of *L. monocytogenes* in foods has become a major concern to food processing industry and government regulatory agencies (Todd,1989). The organism is able to grow under a variety of conditions, especially at refrigerated temperatures (Shelef,1989). In addition, *L. monocytogenes* is able to survive at high-temperatured and short-timed pasteurization and in acid conditions with pH as low as 4.8 in foods (Doyle *et al.*, 1987). The results of this study revealed that the use of bacteriocin produced by *P. acidilactici* in combination with traditional methods such as refrigeration, pasteurization, and low pH could be effective in controlling *L. monocytogenes* on food products.

Nature of the bacteriocin and their mode of action

To identify the mode of action, changes in

Table 1 Inhibitory spectrum of *P. acidilactici* isolated from nham.

Indicator strain	Sensitivity*	Source**
Gram positive bacteria		
Listeria monocytogenes	++	FDA
Listeria innocua	+	FDA
Listeria ivanovii	++	FDA
Clostridium perfringens	+	IFRPD
Staphylococcus aureus	+	IFRPD
Bacillus cereus	++	IFRPD
Enterococcus faecalis	++	IFRPD
Gram negative bacteria		
Salmonella enteritidis	-	IFRPD
Salmonella derby	-	IFEPD

* Sensitivity was measured by the spot-on-lawn technique, 5 l of 60% ammonium sulphate fraction was assayed on lawn of test bacteria

Symbols : - not inhibited
+ weakly inhibited (10 to 18 mm in diameter)
++ highly inhibited (> 18 mm in diameter)

** FDA : US Food and Drug Administration
IFRPD : Institute of Food Research and Product Development, Kasetsart University, Thailand.

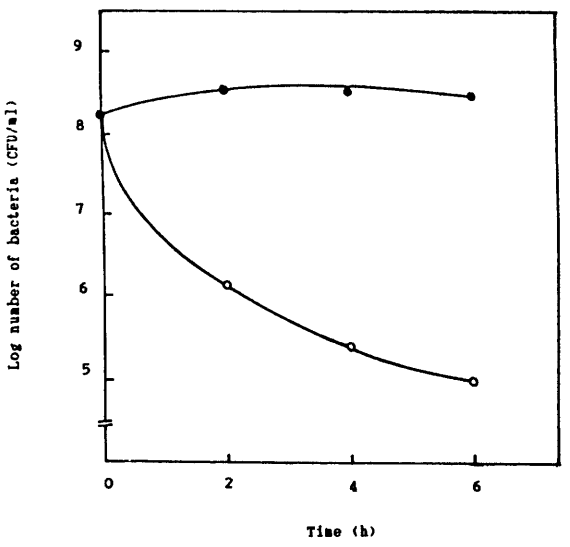

Figure 1 Bactericidal effect of the inhibitor produced by P. acidilactici. Bacteriocin partial purified by ammonium sulphate precipitation was added at concentrarion of 0 (l) and 200 (¡) AU/ml to a culture of indicator strain.

viable counts of the indicator strain were measured (Figure 1). By addition of the inhibitor solution (200 AU/ml) to log-phase cells, after 2h, great decrease in the viable counts was obtained. The results hereby suggested bactericidal effect of the inhibitor.

The inhibitor was completely inactivated by trypsin (Table 2). This fact indicated that the inhibitor was proteinaceous in nature.

From the broad inhibitory spectrum, bactericidal mode of action, and proteinaceous nature, the inhibitor produced by *P. acidilactici* isolated from nham was concluded to be a bacteriocin.

No significant differences in activity were observed in the stability of the bacteriocin during storage for 6h at pH values in the range of 3-10. Similar results were found for other bacteriocins produced by lactic acid bacteria (Bhuniaetal., 1988). This stability, over a wide range of pH values, could be useful when this bacteriocin used as an antibacterial agent in food

Table 2 Residual activity of the inhibitor produced by P.acidilactici after various treatments.

Treatments	Residual Activity (%)*
Heat - treatments	
60°C 60 min	90
80°C 60 min	80
100°C 30 min	70
Autoclaved (121°C 15 min)	0
At various pH	
pH 3.0	100
4.0	100
5.0	100
6.0	100
7.0	95
8.0	95
9.0	95
10.0	95
Enzyme	
Trypsin	0
Detergents (0.5%)	
Triton x-100	90
Tween 80	90
SDS	80
Control (no treatment)	100

* Activity was measured by the spot-on-lawn technique, 5l of 60% ammonium sulphate fraction was assayed on lawn of indicator strain *(L.plantarum)*

products.

The bacteriocin was highly stable when subjected to heat treatments with temperatures in the range of 60-100°c. The inhibitory activity was completely lost after autoclaved at 121°C for 15 min. Heat-stable has been reported for several other bacteriocins produced by Lactobacillaceae (Klaenhammer,1988).

It is a desirable idea to preserve food by natural means, and so is true for today's consumers who are reluctant to purchase foods containing chemical preservatives. The lactic acid and concommitant production of bacteriocins by many lactic starter culture may provide safe and rather natural way of food preservation as well as controlling health hazardous food spoilage and pathogenic bacteria.

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