Effect of Glutathione on Bacteriocins of Lactic Acid Bacteria Isolated from Traditional Thai Fermented Meat

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Abstract—**An in-vitro enzymatic reaction between glutathione (50, 125 and 250 mM) and various bacteriocins produced by *Pediococcus pentosaceus* TISTR 536 (pediocin PA-1, isolated from traditional Thai fermented meat), *Lactobacillus plantarum* NF3 (plantaricin W, isolated from traditional Thai fermented fish), *Lactococcus lactis* subsp. *lactis* N12 (nisin Z, isolated from traditional Thai fermented rice noodle) compared with *Lactococcus lactis* subsp. *lactis* Sb2 (nisin A, isolated from fish gastrointestinal tract) was examined. The products of the reaction were analyzed by antibacterial – activity assays. The results indicated that nisin A and nisin Z was inactivated by an enzymatic reaction with 250 mM glutathione for 12 h under room temperature (30-32°C). In contrast, glutathione in each studied concentration for 24 h showed no effect on reducing the activity of pediocin PA-1 and plantaricin W under room temperature and 4 °C. This study implies the possibility of using pediocin PA-1 and plantaricin W together with lactic acid for increasing an effective in pathogen inhibition and microbiological control of fresh meat in order to prolong the shelf life and safety quality of fresh meat for consumer**

Keywords—**Glutathione, Bacteriocin, Lactic acid bacteria, traditional Thai fermented meat**

I. INTRODUCTION

Bacteriocins from lactic acid bacteria (LAB) have attracted special interests from the aspect of their potential use as safe and natural food preservatives (biopreservatives) and antimicrobials [1]. Isolated LAB from fermented food and animal gastrointestinal tract have been reported to produce varied bacteriocins such as pediocin PA-1(from *Pediococcus pentosaceus* TISTR 536) isolated from traditional Thai fermented meat product (Nham) [2]**,** plantaracin w (from *Lactobacillus plantarum* NF3) isolated from traditional Thai fermented fish (Nham-pla**)** [3] , nisin Z (from *Lactococcus lactis* subsp. *lactis* N12) isolated from traditional Thai fermented rice noodle (Kanom jien) [4] and nisin A (from *Lactococcus lactis* subsp. *lactis* Sb2) isolated from fish gastrointestinal tract. [5] etc. These bacteriocins implied to inhibit mostly gram positive bacteria especially pathogenic bacteria such as *Staphylococcus aureus*, *Listeria monocytogenes* etc. Moreover, pediocin PA-1 has also been reported to inhibit gram negative bacteria such as *Salmonella* Anatum during these cells were injured under low pH condition [6].

[7] reported their hypothesized that nisin can be inactivated in fresh meat by an enzymatic reaction with glutathione (GSH). Glutathione [N-(N-L-γ-glutamyl-Lcysteinyl) glycine] is a major low molecular weight (307 Da) thiol compound found in cells . GSH is widespread in nature and is found in

both plant and animal tissues. Considering that GSH is an abundant thiol compound in animal tissues and that sulfhydryl groups are a known target of nisin. It seems likely that nisin is easily intercepted by GSH, as was suggested by [8]. Since we plan to provide various bacteriocins to inhibit various pathogenic bacteria concerned in meat and meat products, therefore, this work is aimed to study enzymatic reaction between glutathione and various bacteriocins of nisin A, nisin Z, pediocin PA-1 and plantaracin W produced from the LAB in our stock cultures. The basic data from this study can be helped us to select the right bacteriocins and bacteriocin-producing strains to control the pathogenic bacteria concerned in meat and meat products.

II. MATERIALS & METHODS

Bacterial strains and media : All bacterial strains used in this study were obtained from the Laboratory of Faculty Agro-Industry, King Mongkut's Institute of Technology Ladkrabang (KMITL), Thailand) as frozen stock cultures at –70 °C in MRS broth with 15 % glycerol **(***Lc. lactis* subsp. *lactis* N12**,** *P. pentosaceus* TISTR 536**,** *Lb. plantarum* NF3 and *Lb. sakei* JCM 1157)**,** MRS + 0.85% NaCl with 15 % glycerol (*Lc. lactis* subsp. *lactis* Sb2) and Tryptic soy broth + 0.6%Yeast extract (TSBYE) with 15 % glycerol (*Listeria innocua* ATCC 33090**).**

Preparation of purified glutathione solution : Stock solutions of purified glutathione(≥ 98% L-Glutathione reduced, Sigma-Aldrich co, Japan) were dissolved in a 50 mM sodium phosphate buffer (pH 6.0). Glutathione was dissolved at concentrations ranging from 50, 125 and 250 mM, and the solution was pH adjusted to 6.0 with 1N NaOH and then filter-sterilized with 0.22 μm pore-size polysulfone [9].

Preparation of bacterial solution : Bacterial strains and media bacteriocin-producing strains used in this study are listed in Table 1. All bacteriocins-producing strains used in this study are stored cultures at -70°C and propagated in MRS broth and MRS+0.85% NaCl broth 0.1 ml at 30 °C for 16-18 h. To obtain bacterial for the examination *Lactobacillus sakei* JCM 1157 and *Listeria innocua* ATCC 33090 from stock cultures were 2 times cultured in MRS broth and TSB+0.6%YE incubated for 18-20 h at 37 °C with an initial load about 10^6 cfu/ml before use.

Stability of the bacteriocins on GSH concentrations in vitro : Stock solutions of bacteriocins and purified glutathione were dissolved in a 50 mM sodium phosphate buffer (pH 6.0). Glutathione was dissolved at concentrations ranging from 50 ,125 and 250 mM, and the solution was pH adjusted to 6.0 with 1N NaOH. A control reaction consisted of the same concentrations of bacteriocins and glutathione in absence of the enzyme. Reaction were carried out at Room temperature (30-32^{o}C) and 4 °C . The reaction was stopped every 6 h (0, 6, 12, 18 and 24 h) by adding trifluoroacetic acid to final concentration of 0.6%. The products were analyzed antibacterial activity as described by [9].

Antimicrobial activity assay : To determine the extent of loss of antimicrobial activity against *Lb. sakei* JCM 1157 and *Lis. innocua* ATCC 33090 using the spot-on-lawn technique with MRS agar and TSBYE agar [10]. was determined every 6 h (0, 6, 12, 18 and 24h). Those samples exhibiting activity were assayed for total activity by serial dilution. Total activity was determined by taking the reciprocal of the highest dilution that gave a clear zone of inhibition and war expressed as arbitrary units per ml (AU/ml).

Table 1. Bacteriocin-producing strains and medium used in this study.

Species	Bacteriocin	Medium	Reference
Lactococcus lactis subsp. *lactis* Sb2	nisin A	MRS+0.85% NaCl	[5]
Lactococcus lactis subsp. *lactis* N12	nisin Z	MRS	[4]
Pediococcus pentosaceus TISTR 536	pediosin PA-1	MRS	[2]
Lactobacillus plantarum NF3	plantaricin W	MRS	[3]

III. RESULTS & DISCUSSION

The products of the reaction of crude bacteriocins from various bacteriocin-producing strains on GSH (50, 125 and 250 mM), were analyzed for antimicrobial activity against *Lb. sakei* JCM 1157 (Table 2). The results indicated that only nisin group of bacteriocins (nisin A and nisin Z) were completely inactivated by an enzymatic reaction with 250 mM GSH for 24 h under room temperature (30-32°C), while the activity of both bacteriocins was decreased from 6,400 AU/ml to 200 AU/ml (nisin A) and to 400 AU/ml (nisin Z) after contacted to GSH under 4 °C for 24 h. The same decreasing results of the activity of nisin A and nisin Z after contacted to GSH under room temperature and 4 °C for 24 h were also exhibited when *Lis. innocua* was used as indicator strain (Table 3). The results from this study were strongly supported the hypothesis of [7] who hypothesized that nisin can be inactivated by an enzymatic reaction with glutathione.

For other studied crude bacteriocins, it was revealed that GSH gave no effect on plantaricin W and pediocin PA-1. The activity of plantaricinW after contacted to GSH in each studied concentration for 24 h showed no effect on reducing under room temperature and 4 °C when *Lb. sakei* was used as indicator strain (Table 2), but exhibited little reduction on activity when *Lis. innocua* was used as indicator (Table 3). The little reduction in activity results of pediocin PA-1 was also revealed on *Lis. innocua* (Table 3), while there was no inhibition activity of peiocin PA-1 on *Lb. sakei* from the beginning of the study (0 h, Table 2). Thus, both of *Lb. plantarum* NF3 and *P. pentosaceus* TISTR 536, which produce plantaricin W and pediocin PA-1 respectively, can be used as starter cultures for microbiological safety during meat fermentation.

Table 2. Antimicrobial activity (AU/ml) of bacteriocins reacted with GSH in vitro by Indicator *L. sakei* JCM 115

Bacteriocins with GSH	**Activity Units (AU/ml)**			
	Room temp		**4 °C**	
	0 h	**24 h**	**0 h**	**24 h**
nisin A + GSH 50 mM	6,400	400	6,400	800
nisin A + GSH 125 mM	6,400	200	6,400	800
nisin A + GSH 250 mM	3,200	0	3,200	200
nisin Z + GSH 50 mM	6,400	1,600	6,400	3,200
nisin Z + GSH 125 mM	6,400	400	6,400	1,600
nisin Z + GSH 250 mM	3,200	0	3,200	400
pediocin PA-1 + GSH 50 mM	0	0	0	0
pediocin PA-1 + GSH 125 mM	0	0	0	0
pediocin PA-1 + GSH 250 mM	0	0	0	0
plantaricin W + GSH 50 mM	12,800	12,800	12,800	12,800
plantaricin W + GSH 125 mM	12,800	12,800	12,800	12,800
plantaricin W + GSH 250 mM	6,400	6,400	6,400	6,400

nisin A = *Lactococcus lactis* subsp. *lactis* Sb2 , nisin Z= *Lactococcus lactis* subsp. *lactis* N12, pediosin PA-1= *Pediococcus pentosaceus* TISTR 536 , plantaricin w = *Lactobacillus plantarum* NF3

Table 3. Antimicrobial activity (AU/ml) of bacteriocins reacted with GSH in vitro by Indicator *L. innocua* ATCC 33090

Bacteriocins with GSH	**Activity Units (AU/ml)**			
	Room temp		**4 °C**	
	0 h	**24 h**	**0 h**	**24 h**
nisin A + GSH 50 mM	3,200	200	3,200	400
nisin A + GSH 125 mM	3,200	0	3,200	0
nisin A + GSH 250 mM	1,600	0	1,600	0
nisin Z + GSH 50 mM	6,400	400	6,400	400
nisin Z + GSH 125 mM	6,400	100	6,400	100
nisin Z + GSH 250 mM	3,200	0	3,200	0
pediocin PA-1+ GSH 50 mM	800	400	800	400
pediocin PA-1+ GSH 125 mM	800	400	800	400
pediocin PA-1+ GSH 250 mM	400	100	400	200
plantaricin W + GSH 50 mM	3,200	400	3,200	1,600
plantaricin W + GSH 125 mM	3,200	200	3,200	1,600
plantaricin W + GSH 250 mM	1,600	200	1,600	800

IV. CONCLUSION

The results from this study imply that, when compared to nisin-producer strains, pediocin PA-1 and plantaricin W producers show the possibility to be used as starter cultures to inhibit the growth of some bacterial pathogens during meat fermentation. Moreover, both pediocin PA-1 and plantaricin W can also be used as biopreservative to control some

bacterial pathogens concerned in fresh meat in order to prolong the shelf life and safety quality of fresh meat for consumer.

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