DETERMINATION OF THE EFFECT OF GLUTATHIONE ON BACTERIOCINS FROM LACTIC ACID BACTERIA USING LC/MS

A. Swetwiwathana[1], Y. Masuda[2], T. Zendo[2], J. Nakayama [2], and K. Sonomoto[2]

[1] Division of Fermentation Technology, Faculty of Agro-Industry, King Mongkut's Institute of Technology Ladkrabang, Bangkok 10520, Thailand. [2] Laboratory of Microbial Technology, Division of Microbial Science and Technology, Department of Bioscience and Biotechnology, Faculty of Agriculture, Graduate School, Kyushu University, Fukuoka 812-8581, Japan

Abstract – The results in this study indicated that only nisin group of bacteriocins were mostly completely inactivated by an enzymatic reaction with 250 mM glutathione (GSH) for 24 h under 30-32 °C. GSH, which is mostly found in both plants and animal tissues, showed no effect on the activity of pediocin PA-1 from *Pediococcus pentosaceus* TISTR 536. LC/MS can be used to confirm the change of molecular mass of purified nisin A after contact with GSH, but is not useful for molecular mass determination of crude bacteriocins in cell free culture supernatants of MRS broth due to protein impurities in MRS broth. The study demonstrated that pediocin PA-1 can be used as biopreservative to control some bacterial pathogens in fresh meat.

Key Words – bacteriocins, lactic acid bacteria, glutathione, meat, Liquid

I. INTRODUCTION

Bacteriocins from lactic acid bacteria (LAB) have attracted special interest from the aspect of their potential use as safe and natural food preservatives (biopreservatives) and antimicrobials. LAB isolated from Thai traditional fermented foods and animal gastrointestinal tract have been reported to produce s variety of bacteriocins such as pediocin PA-1 from *Pediococcus pentosaceus* TISTR 536 isolated from traditional Thai fermented meat product (Nham) [1], nisin Z from *Lactococcus lactis* subsp. *lactis* N12 isolated from traditional Thai fermented rice noodle (Kanom jien) [2] and nisin A produced by *Lactococcus lactis* subsp. *lactis* Sb2 isolated from the fish gastrointestinal tract [3]. These bacteriocins inhibit mostly gram positive bacteria especially pathogenic bacteria such as *Staphylococcus aureus* and *Listeria monocytogenes*. Moreover, pediocin PA-1 has also been reported to inhibit gram negative bacteria such as *Salmonella* Anatum when these cells are injured under low pH [4].

Rose et al. [5] hypothesized that nisin can be inactivated in fresh meat by an enzymatic reaction with glutathione (GSH). Glutathione [N-(N-L-γ-glutamyl-Lcysteinyl) gly-cine] 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 Gross and Morell [6]. The reaction of glutathione extracted from beef with nisin, which changed the structures and reduced the antimicrobial activity of nisin, had already been proved by using MALDI-TOF mass spectrophotometer [7]. Since we plan to provide different bacteriocins to inhibit various pathogenic bacteria found in meat and meat products, Tilokavichai et al. [8] studied the effect of various concentrations of glutathione (50, 125 and 250 mM) on the activity of nisin A and Z. They reported that the activity of nisin A and nisin Z was totally inactivated by an enzymatic reaction with 250 mM glutathione for 12 h at room temperature (30-32°C). In contrast, glutathione in each studied concentration for 24 h showed no effect on the activity of pediocin PA-1 and plantaricin W at room temperature and 4°C. This work aimed to confirm the enzymatic reaction between glutathione and various crude bacteriocins of nisin A, nisin Z, and pediocin PA-1 produced from the LAB in our stock cultures and to monitor the changes in molecular mass using LC/MS [2, 9]. The basic data from this study can help us to select the right bacteriocins and bacteriocin-producing strains to control pathogenic bacteria in meat and meat products.

II. MATERIALS AND METHODS

Bacterial strains and media:
Bacteriocin-producing strain of *Lactococcus lactis* subsp. *lactis* N12 (nisin Z)**,** *Lc. lactis* subsp. *lactis* Sb2 (nisin A) and *P. pentosaceus* TISTR 536 (pediocin PA-1) and *Lb. sakei* JCM 1157 (indicator) were cultured in MRS broth at 30-32°C for 20 h. *Listeria innocua* ATCC 33090 (indicator) was cultured in Tryptic soy broth + 0.6%Yeast extract (TSBYE) at 30-32°C for 20 h. Each strain was subcultured twice prior to use in the study. Purified nisin A purchased from Sigma was used as a positive control.

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 concentration 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 [8].

Stability of the bacteriocins on GSH concentrations in vitro:
Crude bacteriocins from the cell free supernatant (CFS) of a 20 h of culture of each bacteriocin producer and purified glutathione were dissolved in a 50 mM sodium phosphate buffer (pH 6.0). Glutathione was dissolved at 250 mM, and the solution was adjusted to pH 6.0 with 1 N NaOH. Control reaction samples consisting of more than 12,800 AU/mL of bacteriocins and 250 mM glutathione, including the control sample in absence of the enzyme were prepared. Reactions between bacteriocins in CFS and purified nisin A at 0 h and 24 h were carried out at room temperature (30-32°C). The reaction of each sample was stopped by adding trifluoroacetic acid to final concentration of 0.6%. The products were analyzed for antibacterial activity as described by Rose et al. [5] and the change of their molecular mass was determined using LC/MS as described by Zendo et al. [9].

III. RESULTS AND DISCUSSION

The products of the reaction of crude bacteriocins (from 20 h cultured of various bacteriocin-producing strains in MRS broth) and the purified commercialized nisin A with GSH (250 mM/mL) were analyzed for antimicrobial activity against *Lb. sakei* JCM 1157. The results indicated that only the nisin group of bacteriocins was inactivated by an enzymatic reaction with 250 mM GSH for 24 h at 30-32°C. The activity of nisin A from *Lc. lactis* subsp. *lactis* Sb2 was reduced from 12,800 AU/mL to 400 AU/mL (Figure 1), while the activity of nisin Z from *Lc. lactis* subsp. *lactis* N12 was completely inactivated after contact with GSH at 30-32°C for 24 h (Figure 2). GSH had no effect on the activity of pediocin PA-1 from *Pediococcus pentosaceus* TISTR 536 (Figure 3). When the commercialized purified nisin A that was used as the positive control to study the effect of GSH (250 mM/mL) of this group of bacteriocins, the results revealed that GSH had the effect on the activity of nisin A exhibited by the reduction of activity from 12,800 AU/mL to 1,600 AU/mL. The results concur with the report of Tilokavichaiet al. [8].

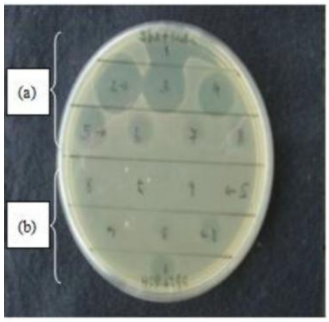

Figure 1. Effect of GSH (250 mM/ml) on activity of crude nisin A from *Lc. lactis* Sb2 after incubated at 30-32 °C for 24 h : (a) crude nisin A, (b) crude nisin A with GSH

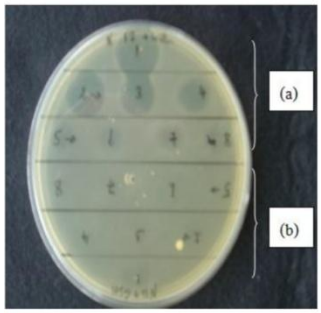

Figure 2. Effect of GSH (250 mM/ml) on activity of crude nisin Z from *Lc. lactis* N12 after incubated at 30-32 °C for 24 h : (a) crude nisin Z, (b) crude nisin Z with GSH

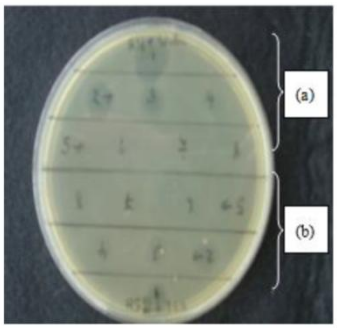

Figure 3. Effect of GSH (250 mM/ml) on activity of crude pediocin PA-1 from *P. pentosaceus* TISTR 536 after incubated at 30-32 °C for 24 h : (a) crude pediocin PA-1, (b) crude pediocin PA-1 with GSH

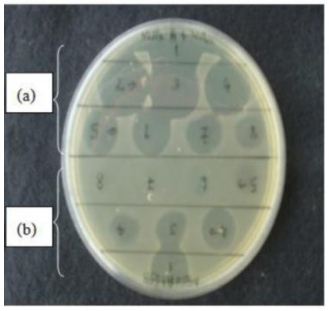

Figure 4. Effect of GSH (250 mM/ml) on activity of purified nisin A after incubated at 30-32 °C for 24 h : (a) purified nisin A, (b) purified nisin A with GSH

For molecular mass study of crude bacteriocins by LC/MS, it was revealed that the molecular mass of purified nisin A was determined by LC/MS around 24 min (Figure 5a). The change in the molecular mass of purified nisin A occurred after contact with 250mM/mL GSH for 24 h (Figure 5b). It can be concluded that GSH has affected the structure of nisin A which may account for the loss of inhibitory activity of nisin A. The results from this study are strongly supported by the hypothesis of Rose et al. [5] who hypothesized that nisin can be inactivated by an enzymatic reaction with glutathione.

When using crude nisin A and Z directly from a 20 h MRS culture of *Lc. lactis* Sb2 and *Lc. lactis* N12, respectively, to determine molecular mass of both nisin A and Z by LC/MS with the same system recommended by Zendo et al. [8], the results revealed that molecular mass of both bacteriocins could not be clearly determined from the chromatogram due to the presence of protein impurities from the MRS broth (Figures 6 & 7). The same observation also occurred in the chromatogram of crude pediocin PA-1 produced by *P. pentosaceus* TISTR 536 in MRS broth (Figure 8). Thus, to study the effect of GSH on the change of molecular mass of bacteriocins produced from the bacteriocin-producing strain in MRS broth by LC/MS, the bacteriocins should be purified before LC/MS analysis.

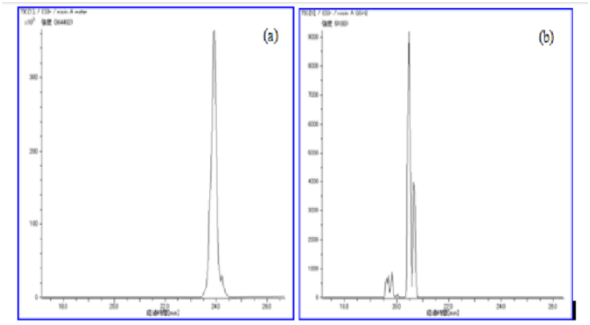

Figure 5. Mass chromatogram extracted the ions with *m/z* 1000-3000 to specify possible bacteriocin-derived peaks. In the mass chromatogram, the retention time of a possible purified nisin A was determined to be 24.00 min (a) the change of mass chromatogram of nisin A + GSH (250 mM/mL) was determined around 21 min (b)

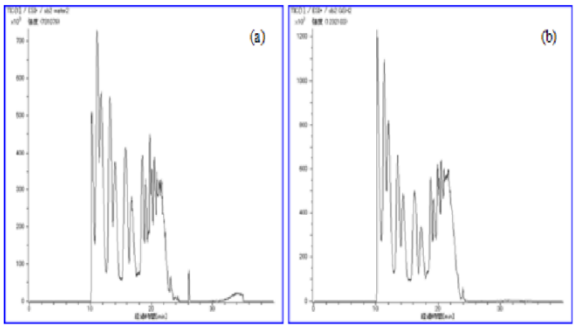

Figure 6. Mass chromatogram extracted the ions with *m/z* 1000-3000 to specify possible bacteriocin-derived peaks (crude nisin A from MRS broth produced by *Lc. lactis* Sb2): a) crude nisin A from MRS broth, b) crude nisin A + 250 mM/mL GSH

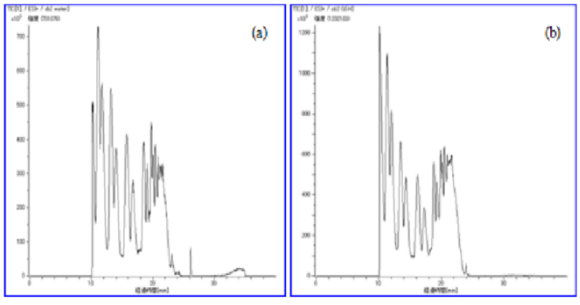

Figure 7. Mass chromatogram extracted the ions with *m/z* 1000-3000 to specify possible bacteriocin-derived peaks (crude nisin Z from MRS broth produced by *Lc. lactis* N12): a) crude nisin Z from MRS broth, b) crudenisin Z + 250 mM/mL GSH

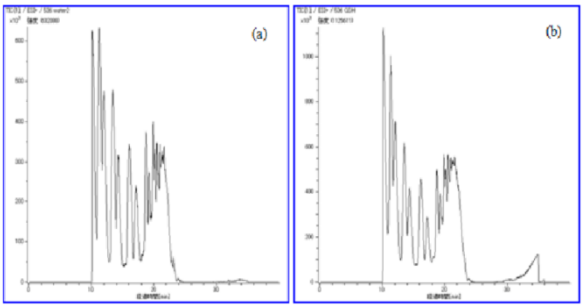

Figure 8. Mass chromatogram extracted the ions with *m/z* 1000-3000 to specify possible bacteriocin-derived peaks (crude pediocin PA-1 from MRS broth produced by *P. pentosaceus* TISTR 536): a) crude pediocin PA-1from MRS broth, b) crude pediocin PA-1 + 250 mM/mL GSH

IV. CONCLUSION

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

ACKNOWLEDGEMENTS

This work was supported in part by the Japan Society for the Promotion of Science (JSPS) and National Research Council of Thailand (NRCT) under Asian Core Program.

REFERENCES

1. Swetwiwathana, A. (2005). Microbiological enhancement of Thai fermented meat product (Nham) using Nham associated pediocin-producing lactic acid bacteria (*Pediococcus pentosaceus* TISTR 536). Ph.D. Thesis of Kyushu University, Japan.

2. Swetwiwathana, A., Zendo, T., Nakayama, J. and Sonomoto, K. (2009). Isolation and identification of Nisin Z producing *Lactococcus lactis* N12. Asian Journal of Food & Agro-Industry 2(02): 116-125.

3. Rumjuankiat, K., Pilasombut, K., Wangwibulkit, S., and Swetwiwathana, A. (2009). Screening and partial characterization of bacteriocin from lactic acid bacteria in fish gastrointestinal tract. Pp. 1-10. in: Proceedings of the 3rd International Conference on Fermentation Technology for Value Added Agricultural Products, August 26-28, 2009; Kosa Hotel, Thailand.

4. Swetwiwathana, A., Lotong, N., Nakayama, J., and Sonomoto, K. (2007). Nham (Thai fermented meat) fermentation: Effect of Pediocin PA-1 producer (*Pediococcus pentosaceus* TISTR 536) as starter culture, nitrite and garlic on Salmonella anatum during Nham (Thai fermented meat) fermentation. Fleischwirtschaft International 3/2007: 46-49.

5. Rose, N.L., Sporns, P., Stiles, M.E., and McMullen, L.M. (1999). Inactivation of nisin by glutathione in fresh meat. Journal of Food Science 64: 759-762.

6. Gross, E., and Morell, J.L. (1971). The structure of nisin. Journal of the American Chemical Society 93: 4634-5.

7. Rose, N.L., Palcic, M.M., Sporns, P., and McMullen, L.M. (2002). Nisin: A novel substrate for glutathione S-transferase isolated from fresh beef Journal of Food Science 67: 2288-2293.

8. Tilokavichai, J., Jindaprasert, A., Pilasombut, K., Sethakul, J., and Swetwiwathana, A. (2011). Effect of glutathione on bacteriocins of lactic acid bacteria isolated from traditional Thai fermented meat. Proceedings (in CD) of the 57th International Congress of Meat Science and Technology (ICoMST). 7-12 August 2011. Ghent, Belgium. P245.

9. Zendo, T., Nakayama, J., Fujita, K. and Sonomoto, K. (2008). Bacteriocin detection by liquid chromatography/mass spectrometry for rapid identification. Journal of Applied Microbiology 104: 499-507.