USE OF PEDIOCIN PA-1 PRODUCER (*PEDIOCOCCUS PENTOSACEUS* TISTR 536) TO CONTROL *SALMONELLA ANATUM* IN NHAM (THAI FERMENTED MEAT)

Adisorn Swetwiwathana*, Napha Lotong**, and Albert Fischer***

* Department of Agro-industry, Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang (KMITL) Bangkok, 10520 Thailand. *Email-address : adisorns@hotmail.com*

** Department of Microbiology, Faculty of Science, Kasetsart University, Bangkok, 10900 Thailand.

*** Department of Meat Technology, Faculty of Food Science and Technology, University of Hohenheim, Stuttgart, Germany.

Background

According to the reports on the advantage of using starter cultures on microbiological quality and safety of Nham production [1, 2] and the recently report on "Potential for Use of Isolated Bacteriocin-Producing *Pediococcus pentosaceus* TISTR 536 from Nham (Thai Fermented Meat) to Control the Growth of *Salmonella anatum* [An In-Vitro Study]" [3], which revealed the advantage of using *P. pentosaceus* strain TISTR 536 as starter culture to control *S. anatum* in created Nham model broth. The bacteriocin from TISTR 536 was later confirmed as pediocin PA-1, which was similar to a bacteriocin of *Pediococcus acidilactici* PAC 1.0 [4, 5]. Since there are a numerous reports on antagonistic substances produced from LAB such as bacteriocins, nisin, pediocin, etc. which exhibited an inhibitory effect on various gram positive microorganisms and sublethal injury cells of various gram-negative bacteria [6, 7]. Thus, it is our aim to rapidly decrease of *S. anatum,* which was the most common contaminated salmonella strain in Nham, during Nham fermentation.

Objectives

This study is to report the use of pediocin producer of *P. pentosaceus* TISTR 536 as starter culture to control *S. anatum* during Nham fermentation compared to the sample of naturally spontaneous fermentation and the sample of using nonbacteriocinogenic *P.pentosaceus* JCM 5890 as starter culture. Besides, effect of pH and Pediocin PA-1 on *S. anatum* in trypticase soy broth (TSB) was also report in this study.

Materials and Methods

Bacterial strains : *P. pentosaceus* strain TISTR 536 [3], *Pediococcus pentosaceus* (JCM 5890), *Listeria innocua* (LTH 3096) and *Salmonella anatum* (obtained from WHO Salmonella-Shigella Center, Bangkok) were used for this study.

Medium : MRS medium modified (Merck), Trypticase soy broth (TSB) (Merck) and TSB + 0.6% yeast extract were used as cultivation medium for each LAB strain, *S. anatum* and *L. innocua* respectively. An overnight cultured of each strain was used for further study. Trypticase soy agar (TSA) was used as medium for *S. anatum* viable cells count. TSA + 0.6% yeast extract and Lactobacillus agar AOAC (Difco) were used for determining of crude Pediocin PA-1 activity from *P. pentosaceus* TISTR 536.

Culture extract preparation and antibacterial determination of TISTR 536

0.1µL of an overnight culture of *P. pentosaceus* strain TISTR 536 was inoculated in 100 mL MRS broth and incubated for 18 h at 35° C. The cultured aliquot was centrifuged at 2,700 x g for 10 min. After centrifugation, the supernatant was adjusted to pH 6.0 with 1.0 N NaOH and filter sterilized with 0.20 µm pore-size polysulfone (Kanto Chemical Co., Tokyo). The crude anti-listerial activity of this cell-free filtrate was determined by the spot-on-lawn method [8] using *L. innocua* LTH 3096 as an indicator strain.

Effect of pH and Pediocin PA-1 on *S. anatum* in trypticase soy broth (TSB)

100 mL of TSB, which the pH was variously adjusted to 6.0, 5.5, 5.0 and 4.5 with 90% lactic acid, was prepared for this study. After a cell concentration of *S. anatum* was added in each TSB sample about 10^4 cells/mL, 2 mL of crude cell-free Pediocin PA-1 (final activity concentration in the each sample was about 256 AU/mL [3]) was added in each TSB sample and incubated at 35° C for 30 h. The viable cells of *S. anatum* in each sample were determined every 6 hours of incubation by pour plate technic with TSA.

Preparation of Nham : The recipe of Nham (650 g minced meat, 350 g shredded cooked pig skin, 60 g cooked rice, 50 g shredded fresh garlic, 25 g salt, 3 g sodium tripolyphosphate, 0.5 g sodium ascorbate, 0.125 g sodium nitrite) was carried out in 1 kg batches per each strain of LAB starter culture and control sample without starter culture. The whole ingredients of samples, except the control sample, were separately inoculated with a strain of *P. pentosaceus* TISTR 536 and JCM 5890 at a level of about 10^6 cfu/g. Approximately 25 g of both Nham control batch mixture without starter culture and Nham with various starter cultures was stuffed into 10 x 14 cm. Plastic bags. All samples were divided into two parts. The first part was examined without inoculating with *S. anatum* and used for determination of pH during Nham fermentation. *S. anatum* was added in the second part of the samples to provide a final concentration ranging from 10 to 10^2 cfu/g. The samples were thoroughly mixed from outside of the bag. The mixture was later squeezed to the closed bag end and tightly wrapped with a rubber band. All samples were left to ferment at room temperature for 6 days. Two bags per sample were examined daily after 3 days of fermentation for determining pH and existence of *S. anatum.*

Microbiological and chemical analysis of Nham

Two bags per sample of Nham were examined daily (0-6 days) for determining of pH and daily after 3 to 6 days of fermentation for existence of *S. anatum* with a standard method for salmonellae detection [2, 9].

Results and Discussion

Effect of pH and Pediocin PA-1 on *S. anatum* in trypticase soy broth (TSB)

The efficacy of pediocin PA-1 (activity was about 256 AU/mL) on *S. anatum* in various pH of TSB was shown in Fig. 1. When compared to TSB broth without crude antagonistic substances, TSB with pediocin PA-1 under pH 5.5 and 6.0 could slightly retard the growth of the studied pathogen. Pediocin PA-1 exerted a better inhibitory effect on *S. anatum* in TSB with pH 5.0, but the best diminishment result was occurred to this studied pathogen within 12 h of incubation when pH of TSB was lower to 4.5. This can be explained in that most of *S. anatum* cells, which are gram negative bacteria and mostly resisted to bacteriocins of gram positive, became injure after survived sublethal in high concentration of weak acid. This synergistic effect of the lower pH and the added pediocin PA-1 in TSB could strongly eradicate these stress cells of *S. anatum.*

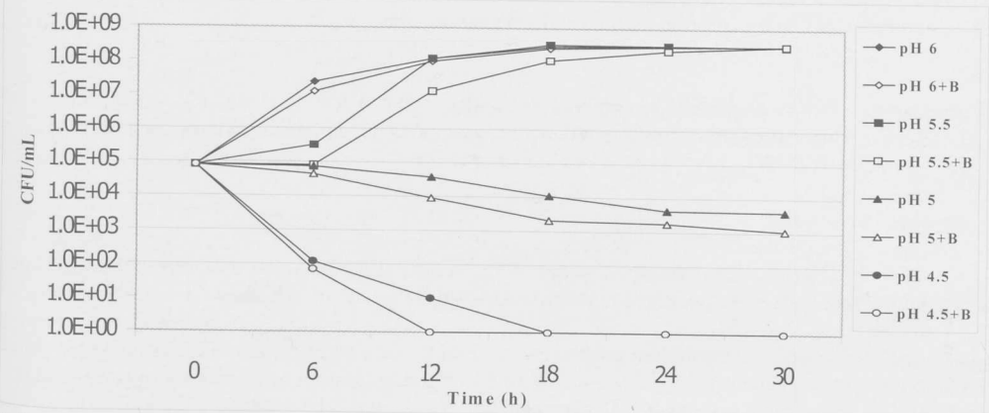
Effect of using pediocin PA-1 producer and non-bacteriocins producer on *S. anatum* during Nham fermentation

The treatment of various amount of *S. anatum* (10, and 10^2 cells/g) with various starter cultures (Pediocin PA-1 producer of *P. pentosaceus* TISTR 536 and non-bacteriocinogenic *P. pentosaceus* JCM 5890) was performed in Nham and compared to those of naturally fermented samples (control without starter culture). Each sample was daily (0-6 days) examined for the reduction of pH and a 25 g portion of each sample after 3-6 days of fermentation was examined for existence of *S. anatum*. The results in Table 1 revealed that the samples of Nham,

which used both of starter cultures, could a bit rapidly decrease in pH of the products during fermentation when compared to the control samples of naturally fermentation. With an existence of *S. anatum* in lower pH under 5.0 for 2-4 days before viable cells count examination might lead the preinoculated *S. anatum* in each sample to cause acid-injured cells and lead these stress cells sensitive to bacteriocins from producer [7].

The results in Table 2 showed the advantage of using both starter cultures in the production of salmonellae free Nham when compared to the samples of naturally fermentation. Among the use of these two studied strains of LAB as starter cultures, pediocin PA-1 producer strain TISTR 536 was found to be the most inhibitory. The results of this study concurred with our earlier report of using the created Nham model broth [3]. In addition, we found that load of *S. anatum* contamination in the product also had an effect in the production of salmonellae free Nham. The lowest amount of preinoculated *S. anatum* (8-10 cfu/g) could rapidly eradicate within 4 and 5 days in sample with pediocin PA-1 producer and sample with nonbacteriocinogenic strain as starter cultures respectively, while the higher load of preinoculated *S. anatum* samples (80-100 cfu/g) took 5-6 days for the elimination. These results were also concurred with many other earlier studied, which reported in the advantage of using starter cultures for a positive effect on the microbiological quality and safety of fermented meat products and the effect of bacteriocins producer on sublethal injury gram-negative bacteria [2, 6, 7, 10].

Figure 1 : Effect of pH and Pediocin PA-1 on *S. anatum* in TSB after 30 h incubation under 35° C



+ B = with pediocin PA-1 (activity about 256 AU/mL)

Table 1 : pH of Nham during 6 days of fermentation

Day	control without starter	Nham with TISTR 536	Nham with JCM 5890
0	6.10	6.10	6.10
1	5.31	5.27	5.28
2	4.84	4.79	4.78
3	4.71	4.65	4.62
4	4.60	4.56	4.54
5	4.49	4.44	4.42
6	4.30	4.25	4.24

Table 2 : Effect of pediocin PA-1 producer (*Pediococcus pentosaceus* TISTR 536) and nonbacteriocinogenic *P. pentosaceus* JCM 5890 on *S. anatum* during Nham fermentation

Sample	*S. anatum* recovered (days)							
	8-10 cfu/g				80-100 cfu/g			
	3	4	5	6	3	4	5	6
Control without starter	+	+	+	+	+	+	+	+
Nham with TISTR 536	+	-	-	-	+	+	-	-
Nham with JCM 5890	+	+	-	-	+	+	+	-

Conclusions

The study reported that synergistic effect of the lower pH and the added pediocin PA-1 in TSB exerted the best inhibitory effect on injured-cells of *S. anatum*. Use of the isolated pediocin PA-1 producing *P. pentosaceus* TISTR 536 from Nham as starter led to faster salmonellae-free Nham production than use of non-bacteriocinogenic strain as starter and naturally fermentation respectively.

References

1. Swetwiwathana, A., U. Leutz, and A. Fischer. 1999. Role of Garlic on Growth and Lactic Acid Production of Starter Cultures. Fleischwirtschaft International. 1(1999) : 26-29. **2. Swetwiwathana, A., U. Leutz, N. Lotong, and A. Fischer. 1999.** Controlling the Growth of *Salmonella anatum* in Nham. Fleischwirtschaft. 79(9) : 124-128. **3. Swetwiwathana, A., N. Lotong, A. Fischer, and K. Sonomoto. 2001.** Potential for Use of Isolated Bacteriocin-Producing *Pediococcus pentosaceus* TISTR 536 from Nham (Thai Fermented Meat) to Control the Growth of *Salmonella anatum* [An In-Vitro Study]. The 47th International Congress of Meat Science and Technology (ICoMST) Proceeding Volume II, August 26th-31st, 2001 Krakow, Poland. 5-P7 : 18-19. **4. Henderson, J.T., A.L. Chopko, and D. van Wassenaar. 1992.** Purification and Primary Structure of the Bacteriocin PA-1 Produced by *Pediococcus acidilactici* PAC 1.0. Arch. Biochem. And Biophys. 295 : 5-12. **5. Marugg, J.D., C.F. Gonzalez, B.S. Kunka, A.M. Lederboer, M.J. Pucci, M.Y. Toonen, S.A. Walker, L.C.M. Zoetmulder, and P.A. Vandenbergh. 1992.** Cloning, Expression, and Nucleotide Sequence of Genes Involved in Production of Pediocin PA-1, a Bacteriocin from *Pediococcus acidilactici* PAC 1.0. Appl. Environ. Microbiol. 58 : 2360-2367. **6. Stevens, K. A., B. W. Sheldon, N. A. Klapes, and T. R. Klaenhammer. 1991.** Nisin Treatment for Inactivation of *Salmonella* Species and Other Gram-Negative Bacteria. Applied and Environmental Microbiology. 57(12) : 3613-3615. . **7. Kalchayanand, N., M. B. Hanlin, and B. Ray. 1992.** Sublethal Injury Makes Gram-negative and Resistant Gram-positive Bacteria Sensitive to the Bacteriocins, Pediocin AcH and Nisin. Letters in Applied Microbiology. 15 : 239-243. **8. Mayr-Harting, A., A.J. Hedges, and R.C.W. Berkeley. 1972.** Methods for Studying Bacteriocins. Meth. Microbiol. 7A : 315-422. **9. Swetwiwathana, A., and A. Bangtrakulnonth. 1996.** Evaluation of Salmosyst Enrichment Procedure Plus Rambach Agar for Detection of Salmonellae in Nham. The 34th Annual Conference Proceeding, Kasetsart University, Thailand. **10. Hammes, W.P., and H.J. Knauf. 1994.**Starter in the Processing of Meat Products. Meat Science. 36 : 155-168.