SYNERGISTIC EFFECT OF PEDIOCIN PA-1 FROM *PEDIOCOCCUS PENTOSACEUS* TISTR 536 AND LACTIC ACID ON *SALMONELLA* ANATUM AND *S.* RATCHABURI IN SIMULATED NHAM (FERMENTED MEAT SAUSAGE) MODEL BROTH

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Abstract – The synergistic effect of lowering the pH of Nham (traditional Thai fermented meat sausage) model broth (NMB) to 4.5 using lactic acid and the addition of crude pediocin PA-1 (activity about 64 AU/ml) produced by *Pediococcus pentosaceus* TISTR 536 on *Salmonella* Anatum *S.* Bangkok and *S.* Ratchaburi in this model broth revealed that most of the preinoculated pathogens became sublethally injured after surviving in a high concentration of weak acid and led the cells sensitive to pediocin PA-1. *S.* Bangkok revealed sensitive to pH 4.5 than *S.* Anatum and *S.* Ratchaburi. This study implied the beneficial of using pediocin PA-1 producing lactic acid bacteria as starter cultures to control *Salmonella* spp. during the fermentation of Nham product.

Key Words – Lactic acid, Pediocin PA-1, *Salmonella* Anatum, *S.* Bangkok, *S.* Ratchabur

I. INTRODUCTION

Nham, a traditional Thai fermented pork, is normally eaten without cooking. *Salmonella anatum*is the strain which is mostly found contaminated in Nham product [1, 2, 3]. It is, therefore, a serious public health concern. Thus, many recent studies were mostly aimed on inhibition of this strain during the product fermentation, especially the use of lactic acid bacterial (LAB) starter culture [2] including bacteriocin-producing LAB [4]. Since various new serovarieties (serovars) of Salmonellae have been found in many countries including in Thailand during the past decade [5] such as in 1972, a new type of Salmonella serovar Bangkok was firstly found in Bangkok, Thailand. This new serovar was reported in causing diarrheal disease and contaminated in food and environment [6]. In 1999, *S.* Ratchaburi found in Ratchaburi province of Thailand is one of among these new serovars. This serovar was found contaminated in food produced by the carrier staffs in the canteen of Ratchaburi provincial Hospital and firstly reported to cause salmonellosis to 11 patients who consumed foods in the canteen in 1999 [7]. Due to the production of fermented foods in Thailand is still largely a traditional art associated with poor hygiene, which might be concerned with salmonellae carriers such as *S.* Bangkok, *S.* Ratchaburi, *S.* Anatum etc. Thus, this study is to investigate the effect of pediocin PA-1 from *P. pentosaceus* TISTR 536 and lactic acid on the survival of *S.* Bangkok and *S.* Ratchaburi which has never been studied in Nham. The study was conducted in the simulated Nham fermentation broth designated Nham model broth (NMB) as reported by Swetwiwathana et al. (1999 and 2007).

II. MATERIALS AND METHODS

Bacterial Strains

Pediococus pentosaceus strain TISTR 536 [4] and *Lactobacillus sakei* subsp. *sakei* JCM 1157 (Japanese Culture of Microorganisms) [obtained from Laboratory of Faculty Agro- Industry, King Mongku's Insitute of technology Ladkrabang (KMITL), Thailand], *Salmonella* Anatum (isolated from Nham and was confirmed the serovareity from WHO Salmonella-Shigrlla Center, Bangkok), *S.* Bangkok and *S.* Ratchaburi (obtained from

WHO Salmonella-Shigrlla Center, Bangkok), were used for this study.

Crude pediocin PA-1 preparation for P. pentosaceus TISTR 536

Preparation for Crude pediocin PA-1 for *P. pentosaceus* TISTR 536 and detection of bacteriocin concentration were followed the method initiated by Swetwiwathana et al. (2007).

Preparation of Salmonellae

The slant cultured of *S.* Anatum, *S.* Bangkok and *S.* Ratchaburi was inoculated in 10 ml TSB (Merck) and incubated at 35 °C for 20-24 h. 1 ml of an overnight culture was again transferred to 10 ml TSB and incubated at 35 °C for 20 h before using in the study.

Study on the effect of crude Pediocin PA-1and pH on S. Anatum, S. Bangkok and S. Ratchaburi in Nham model broth (NMB)

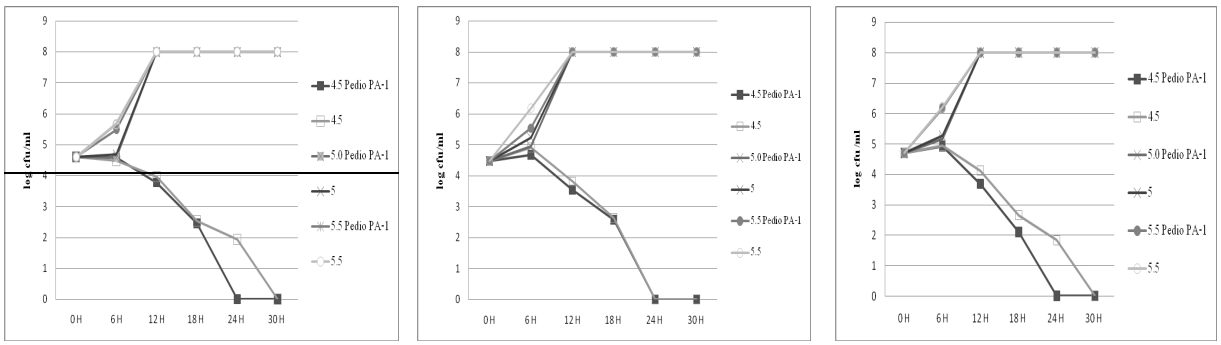
Two sets of 100 ml of NMB was prepared and adjusted pH to 4.5, 5.0 and 5.5 with 90% lactic acid. One set of various studied pH of NMB were added with 2% of sterile crude pediocin PA-1 (activity was about 3,200 AU/ml) which led the final pediocin PA-1 activity in NMB was about 64 AU/ml as described by Swetwiwathana et al. (2007), while the other set of NMB without crude pediocin PA-1 were used as the control set. Each salmonellae strain was later inoculated in both sets of NMB (with an initial load of 10^5 CFU/ml in each broth). All NMB samples were incubated under room temperature (32-34 ^oC) for 30 h. Each NMB sample was determined for available cell count on Trypticase soy agar (TSA) and Xylose Lysine Desoxycholate (XLD) agar at every 6 h interval of incubation. All incubating inoculated plates were counted for viable cell colonies after 20-24 h of incubation at 37^oC. The percentage of salmonella injury cells during the 30 h of incubation was calculated as follows :

$$\% \text{ Injury cells} = \left| 1 - \frac{\text{number of cell counts in XLD}}{\text{number of cell counts in TSA}} \right| \times 100$$

III. RESULTS AND DISCUSSION

Effect of Pediocin PA-1 and pH on S. Anatum, S. Bangkok and S. Ratchaburi in NMB

Crude pediocin PA-1, which showed the activity about 3,200 AU/ml on *Lb. sakei* subsp. *sakei* JCM 1157 (data not shown), implied to have the effect on *S.* Anatum, *S.* Bangkok and *S.* Ratchaburi in Nham model broth (NMB) after 30 h of incubation under pH of this NMB was lowering to 5.5, 5.0 and 4.5 respectively by using 90% lactic acid (Fig. 1). The results revealed that crude pediocin PA-1 in both pH 5.5 and 5.0 showed no effect on *S.* Anatum, *S.* Bangkok and *S.* Ratchaburi. All studied pathogenic salmonellae serovars could grow upto 10^8 cells/ml within 12 h under these 2 studied pH. 64 AU/ml of crude pediocin PA-1 exhibited the best effect on all studied salmonellae pathogens (initial load of 10^5 CFU/ml) in NMB under pH 4.5 within 24 h of incubation. *S.* Bangkok was implied sensitive to lactic acid under pH 4.5 due to the strain could be diminished in NMB without crude pediocin PA-1 within 24 h of incubation, while both *S.* Anatum and *S.* Ratchaburi in NMB without crude pediocin PA-1 were diminished under pH 4.5 within 30 h of incubation. The results were concurred to the report of Swetwiwathana et al. (2007) which informed the best efficacy of pediocin PA-1 (activity was about 256 AU/ml) on *S.* Anatum in TSB at pH 4.5 within 12 h. But the period used for both salmonella serovars diminishment in this study was about 24 h. These might be due to the concentration of crude pediocin PA-1 activity in this study was lower (64 AU/ml) and incubating temperature in this study was 2-4 ^oC (32-34 ^oC) higher than the former study (30 ^oC) which led all studied salmonella rapidly growth during NMB incubation.



(a) (b) (c)

Figure 1 Effect of Pediocin PA-1 (64 AU/ml) and pH on *S*. Anatum (a) *S*. Bangkok (b) and *S*. Ratchaburi (c) in NMB

Table 1 Percentage of injury *S.* Anatum cells in NMB (pH 4.5) with and without crude pediocin PA-1 (64 AU/ml) at different periods

Incubation time (h)	*S*. Anatum cell in NMB without pediocin PA-1			*S*. Anatum cell in NMB with pediocin PA-1		
	TSA (cfu/ml)	XLD (cfu /ml)	Injury cells (%)	TSA (cfu/ml)	XLD (cfu /ml)	Injury cells (%)
0	25000	23800	4.8	25000	23800	4.8
6	30000	15800	47.2	24500	11400	53.3
12	30000	14500	51.2	50000	20000	60.8
18	8100	2400	70.2	85000	22000	74.1
24	27000	5000	80	0	0	0
30	0	0	0	0	0	0

Table 2 Percentage of injury *S.* Bangkok cells in NMB (pH 4.5) with and without crude pediocin PA-1 (64 AU/ml) at different periods

Incubation time (h)	*S*. Ratchaburi cell in NMB without Pediocin PA-1			*S*. Ratchaburi cell in NMB with Pediocin PA-1		
	TSA (cfu/ml)	XLD (cfu /ml)	Injury cells (%)	TSA (cfu/ml)	XLD (cfu /ml)	Injury cells (%)
0	30000	28900	3.6	30000	28900	3.6
6	30000	23600	83.7	30000	17900	86.5
12	17750	5050	71.5	9000	1000	88.2
18	800	0	100	450	0	100
24	0	0	0	0	0	0
30	0	0	0	0	0	0

Table 3 Percentage of injury *S.* Ratchaburi cells in NMB (pH 4.5) with and without crude pediocin PA-1 (64 AU/ml) at different periods

Incubation time (h)	*S*. Ratchaburi cell in NMB without Pediocin PA-1			*S*. Ratchaburi cell in NMB with Pediocin PA-1		
	TSA (cfu/ml)	XLD (cfu /ml)	Injury cells (%)	TSA (cfu/ml)	XLD (cfu /ml)	Injury cells (%)
0	60000	58000	3.3	60000	58000	3.3
6	50000	20000	60.8	11000	3500	67.7
12	27000	5000	82.5	9000	1000	88.2
18	30000	3000	90	5000	0	100
24	20000	1000	95	0	0	0
30	0	0	0	0	0	0

The best diminishment of *S.* Anatum, *S.* Bangkok and *S.* Ratchaburi in NMB with crude pediocin PA-1 under pH. 4.5 can be explained in that *Salmonella* spp., which are gram negative bacteria and mostly resistant to bacteriocins of gram positive, became sublethally injured after surviving in high concentrations of weak acid (Table 1-3) [8]. Thus, this synergistic effect of the lower pH and the added of pediocin PA-1 in NMB led to rapidly eradicated of the stressed cells of all studied salmonella serovars in this study (Swetwiwathana et al., 2007).

IV. CONCLUSION

The study informs the advantage of crude pediocin PA-1 from *P.* pentosaceus TISTR 536 to inhibit *S.* Anatum, *S.* Bangkok and *S.* Ratchaburi under pH 4.5 in simulated Nham model broth. This pediocin PA-1 producer strain can be applied as starter cultures for improving the microbiological quality and safety of Nham, a traditional Thai fermented meat production.

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