

Effect of pediocin PA-1, pH and nitrite on *Salmonella* Anatum and *S. Ratchaburi* in simulated Nham (traditional Thai 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 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. Sodium nitrite (100 ppm), which is normally used as food additive in the production of Nham, exerted to retard the growth of both studied salmonella serovars in NMB during the first 12 h of incubation under room temperature. This study implied the beneficial of using pediocin PA-1, nitrite and pediocin PA-1 producing lactic acid bacteria as starter cultures to control *Salmonella* spp. during the fermentation of Nham product.

Key Words : pediocin PA-1, pH, nitrite, *Salmonella* Anatum, *S. Ratchaburi*

Introduction

Nham ,a traditional Thai fermented pork ,is normally eaten without cooking. *Salmonella anatum*, which is mostly found contaminated in Nham product (Lotong and Swetwathana, 1990; Swetwathana et al., 1994; Swetwathana and Bangtrakulnonth, 1996). 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 (Swetwathana et al., 1999) including bacteriocin-producing LAB (Swetwathana et al., 2007). Since various new serovarieties (serovars) of *Salmonellae* have been found in many countries during the past decade (Popoff and Le Minor, 2002), *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 (Bangtrakulnonth et al., 1999). 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. Ratchaburi*, *S. Anatum* etc. Thus, this study is to investigate the effect of pediocin PA-1 from *P. pentosaceus* TISTR 536, pH and nitrite on the survival of *S. Ratchaburi* which has never been studied in Nham. The study is also compared all effects on *S. Anatum* which was isolated from Nham. The study was conducted in the simulated Nham fermentation broth designated Nham model broth (NMB) as reported by Swetwathana et al. (1999 and 2007).

Materials and Methods

Bacterial Strains

Pediococcus pentosaceus strain TISTR 536 (Swetwathana, 2007) 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 (KMIL), Thailand], *Salmonella* Anatum (isolated from Nham and was confirmed the serovareity from WHO Salmonella-Shigrla Center, Bangkok) and *S. Ratchaburi* (obtained from WHO Salmonella-Shigrla Center, Bangkok), were used for this study.

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

P. pentosaceus TISTR 536 (deep-freeze culture in 15 % glycerol) was prior inoculated in 10 ml MRS broth (Merck) and incubated at 35°C for 20-24 h. One percent of overnight culture was transferred into 100 ml MRS broth and incubated at 35°C 24 h. The culture was centrifuged 5000 rpm at 4°C for 10 min. After centrifugation, the supernatant was filter-sterilized with 0.20 µm pore-size polysulfone (Kanto Chemical Co., Japan). The cell free supernatant (crude pediocin PA-1) was test activity by spot-on-lawn method using *Lb. sakei* subsp. *sakei* JCM 1157 (Swetwathana et al.,2007).

Preparation of Salmonellae

The slant cultured of *S. Anatum* 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-1 and pH on *S. Anatum* and *S. Ratchaburi* in Nham model broth

100 ml of Nham model broth (NMB) (Swetwathana et al., 2007) was prepared and adjusted pH to 4.5, 5.0, 5.5 with 90% lactic acid. 2% of sterile crude pediocin PA-1 (activity was about 3,200 AU/ml) was added into each pH of NMB (final pediocin activity in NMB was about 64 AU/ml). Then 20 h cultured of each *S. Anatum* and *S. Ratchaburi* was transferred to various studied condition of NMB with an initial loaded about 10⁴ cells/ml and incubated at room temperature (32-34°C) for 30 h. Viable cell numbers of each studied *Salmonella* serovar in NMB under pH 4.5 were determined every 6 h using pour plate technique with Trypticase soy agar (TSA) and Xylose lysine desoxycholate (XLD) agar. All incubating inoculated plates were counted for viable cell colonies after 20-24 h of incubation at 37°C. The percentage of salmonella injury cells during the 30 h of incubation was calculated as follows :

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

Study on the effect of nitrite on *S. Anatum* and *S. Ratchaburi* in NMB

100 ml of NMB was prepared and adjust pH to 6.0 with 90% lactic acid. The using sodium nitrite (final concentration was 100 ppm in NMB) as food additive by filter-sterilization was used for studying the effect of nitrite on the growth of each studied salmonellae variety at the level of 10⁴ cells/ml in NMB. All samples of each NMB were left to incubate at room temperature for 30 h. Viable cell numbers of each studied *Salmonella* serovariety in various studied conditions of NMB were determined every 6 h using pour plate technique with Trypticase soy agar (TSA). All incubating inoculated plates were counted for viable cell colonies after 20-24 h of incubation at 37°C.

Results and discussion

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

The effect of crude pediocin PA-1 (activity about 1,600 AU/ml) on *S. Anatum* and *S. Ratchaburi* after 30 h of incubation (Figure 1) revealed that crude pediocin PA-1 in both pH 5.5 and 5.0 showed no effect on *S. Anatum* and *S. Ratchaburi*. Both pathogenic salmonellae serovars could grow upto 10⁸ cells/ml within 12 h, while crude pediocin PA-1 exhibited the best effect on both *S. Anatum* and *S. Ratchaburi* in NMB under pH 4.5. It was found that the best diminishment results occurred to both pathogenic salmonellae serovars within 24 h in NMB with 64 AU/ml of crude pediocin PA-1, while both of salmonella serovars were eradicated in NMB without crude pediocin PA-1 within 30 h at room temperature incubation. The results were concurred to the report of Swetwathana 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 °C higher than (32-34 °C) the former study which led both studied salmonella rapidly growth during NMB incubation.

The best diminishment of *S. Anatum* 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 and 2) [Kalchayanand et al., 1992]. 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 both studied salmonella serovars (Swetwathana et al., 2007).

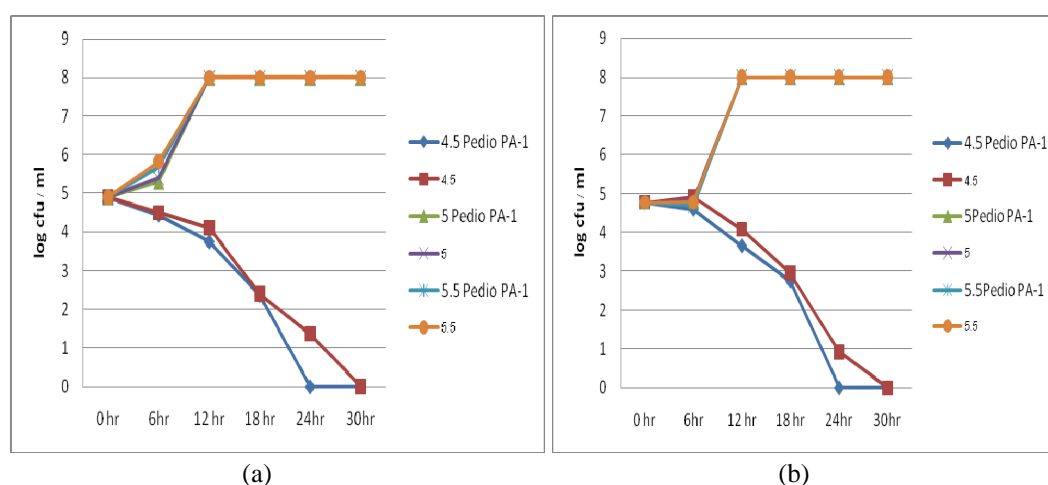


Figure 1 : Effect of Pediocin PA-1 and pH on *S. Anatum* (a) and *S. Ratchaburi* (b) in NMB

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)	<i>S. Anatum</i> cell in NMB without pediocin PA-1			<i>S. Anatum</i> 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. Ratchaburi* cells in NMB (pH 4.5) with and without crude pediocin PA-1 (64 AU/ml) at different periods

Incubation time (h)	<i>S. Ratchaburi</i> cell in NMB without Pediocin PA-1			<i>S. Ratchaburi</i> 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	300000	30000	90	5000	0	100
24	20000	1000	95	0	0	0
30	0	0	0	0	0	0

Effect of nitrite on *S. Anatum* , *S. Ratchaburi* in NMB

The treatment of *S. Anatum* and *S. Ratchaburi* with 100 ppm filter-sterilized sodium nitrite in NMB incubated at room temperature for 24 h was studied and compared to NMB without nitrite (Figure 2). The results revealed that 100 ppm of nitrite in NMB exerted an inhibitory effect on the growth of both salmonella serovars during the first 12 h of NMB incubation. After 12 h of NMB incubation, both pathogens could grow in the medium. This might be clearly explained by the fact that after adding the sterilized sodium nitrite in NMB, undissociated nitrous acid, which was the active inhibitor for various bacteria (Sinskey, 1979), was formed and

retarded the growth of both salmonella serovars. The results were also concurred to the report of Swetwiwathana et al. (2007), but the inhibition period in this study was shorter due to the concentration use of sterilized sodium nitrite was lower (100 ppm) than the early report (125 ppm).

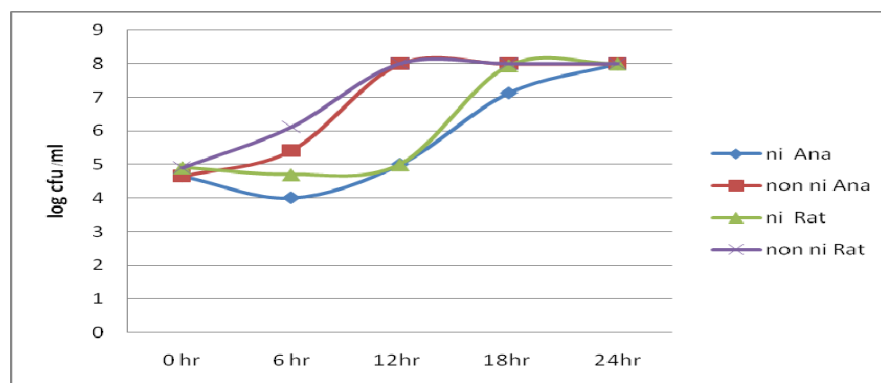


Figure 2 : Effect of nitrite (ni = 100 ppm of nitrite added in NMB, non ni = NMB without nitrite) on *S. Anatum* (Ana) and *S. Ratchaburi* (Rat)

Conclusions

The study informs the advantage of crude pediocin PA-1 from *P. pentosaceus* TISTR 536 to inhibit *S. Anatum* and *S. Ratchaburi* under pH 4.5 in simulated Nham model broth. Nitrite uses in the production of Nham implied to retard the growth of both salmonella pathogens during the early state of Nham fermentation. This pediocin PA-1 producer strain can be applied as starter cultures for improving the microbiological quality and safety of this traditional Thai fermented meat production.

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