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]

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

* 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.
****Laboratory of Microbial Technology, Division of Microbial Science and Technology, Department of Bioscience and Biotechnology, Faculty of Agricultural Graduate School, Kyushu University. 6-10-1 Hakozaki, Higashi-ku, Fukuoka 812-8581, Japan.

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Background

Nham, traditional Thai fermented pork, is normally consumed without cooking and considered as a ready-to-eat food after 3-4 days of spontaneous fermentation. The reports on occurrence of the most common contaminant strain of *Salmonella anatum* in the product [1, 2, 3] are therefore a serious public health concern. Since the advantage of using lactics starter cultures was reports as having a positive effect on the microbiological quality and safety of various fermented products [4, 5], thus, the use of lactics starter cultures to control *S. anatum* in Nham was studied [1, 6]. The selection of the most potent bacteriocin-producing lactic acid bacteria (LAB) from the spontaneous fermentation of this thai fermented meat product had been studied and reported [7, 8]. Among this selected bacteriocin-producing LAB, the strain of TISTR 536 was identified as *Pediococcus pentosaceus*. The strain was also reported to be thermotolerant bacteriocin-producing strain and exert the best antimicrobial spectra of their produces on various indicators, included the opportunistic food pathogens of *Listeria monocytogenes* and *Enterococcus faecalis* [8]. The produced of this strain exhibited no effect on both of *Staphylococcus carnosus* and *Micrococcus varians*, which may enhance adverse effects on colour and aroma of Nham.

Objective

According to the reports on antagonistic substances produced from LAB such as bacteriocins, nisin, pediocin, etc. which exhibited an inhibitory effect on sublethal injury cells of various gram-negative bacteria, especially salmonellae [9, 10]. Thus, this report was conducted to study the potential of using naturally occurring bacteriocin-producing *P. pentosaceus* TISTR 536 from Nham as starter culture to control *S. anatum* in a homogeneous created Nham model broth [6, 11] compared to nonbacteriocin-producer.

Materials and Methods

Bacterial strains : *P. pentosaceus* strain TISTR 536 [7], *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) and Trypticase soy broth (TSB) (Merck) were used as cultivation medium for each LAB strain and *S. anatum* respectively. An overnight cultured of each strain was used for further study.

Nham Model Broth (NMB), which simulated the conditions of Nham production (a_w 0.970, pH 6.3, microaerophilic condition with steriled paraffin oil, added with 125 ppm of filter-sterilized sodium nitrite) and 5 % steriled fresh garlic [6, 11] were used as a model broth for studying the effect of bacteriocin-producing LAB (TISTR 536) and non-bacteriocin-producing LAB of *P. pentosaceus* (JCM 5890) on the growth of *S. anatum* instead of Nham product in this study.

Study on the effect of bacteriocin-producing and non-bacteriocin-producing LAB on the growth of *S. anatum* in the model broth without nitrite and garlic : In order to study the effect of selected LAB on the growth of *S. anatum*, each bacteriocin-producing LAB (TISTR 536) and non-bacteriocin-producing LAB (JCM 5890) at a level of 10^6 cfu/ml was prier studied for their inhibitory effect on *S. anatum* at the level of 10^4 cfu/ml in NMB without nitrite and fresh garlic. The samples of each studied LAB in NMB were left to ferment at 30° C for 2 days after LAB and *S. anatum* inoculation. Analysis of pH (pH Meter SUNTEX model sp-701), bacteriocin activity from the crude culture of each LAB on *L. innocua* (using agar spot assay [12]) and the growth of *S. anatum* (using spread plate technique on trypticase soy agar) were determined every 6 hours of incubation.

Study on an interactive effect of bacteriocin-producing and non-bacteriocin-producing LAB, nitrite and fresh garlic on the growth of *S. anatum* in the model broth : The same volume of each studied strain of LAB and *S. anatum* as described above was conducted in NMB with 125 ppm of filter-steriled nitrite plus 5 % of steriled fresh garlic and left to ferment at 30° C for 2 days. All samples were also determined for pH, bacteriocin activity and the growth of *S. anatum* at the same interval of incubation.

Results and Discussion

Effect of bacteriocin-producing and non-bacteriocin-producing LAB on the growth of *S. anatum* in the model broth without nitrite and garlic

The study of using bacteriocin-producer and nonbacteriocin-producer of *P. pentosaceus* to control the growth of *S. anatum* was prior conducted in NMB without nitrite and fresh garlic under the same condition as Nham production. The results (Fig. 1) revealed that both of study LAB strain and bacteriocin from TISTR 536 exhibited no effect on the growth of *S. anatum*, although the bacteriocin-producer could produce high amount of inhibitory substances (Table 1) and lactic acid (data from decreasing in pH of NMB in Fig. 2). This can be explained that the gram-negative strain of *S. anatum* is not sensitive to the antagonistic produced from TISTR 536 and can growth in a high number during the first day of fermentation. Due to the highly decrease of pH after a day of fermentation, all studied LAB could reduce the cell number of this pathogen in NMB after one day of fermentation which concurred with the earlier report of Swetwiwathana *et al.* [6].

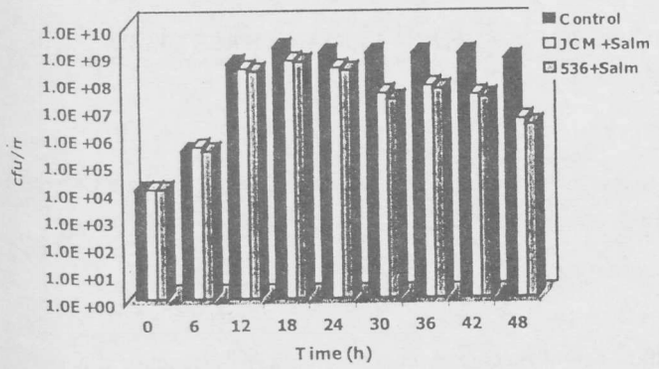

Figure 1 : Effect of 10^6 cfu/ml bacteriocin-producer (TISTR 536) and nonbacteriocin- nonbacteriocin-producer (JCM 5890) on the growth of 10^3 cfu/ml of *S. anatum* (Salm) NMB without nitrite and fresh garlic.

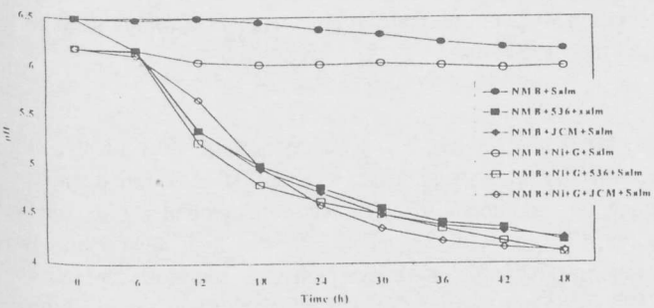

Figure 2 : The reduction of pH in Nham model broth (NMB) with and without nitrite (Ni) and fresh garlic (G) after inoculating with 10^6 cfu/ml of *P. pentosaseus* TISTR (536), 10^6 cfu/ml of JCM 5890 (JCM) and 10^3 cfu/ml of *S. anatum* (Salm)

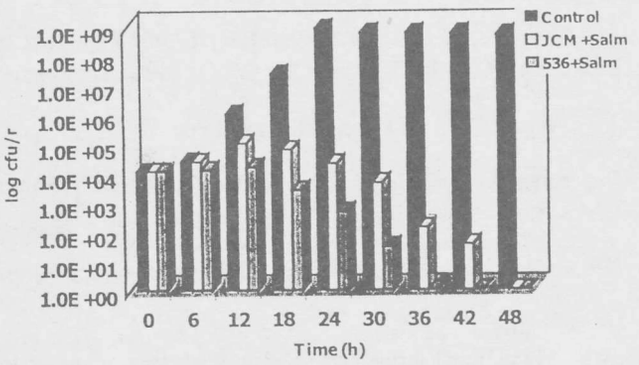

Figure 3 : Interactive effect of 10^6 cfu/ml of bacteriocin-producer (TISTR 536) and nonbacteriocin-producer (JCM 5890), nitrite and fresh garlic on the growth of 10^3 cfu/ml 10^3 cfu/ml of *S. anatum* (Salm) in NMB

Table 1 : Sensitivity in AU/ml of *L. innocua* against antibacterial compound produced by an initial of 10^6 cfu/ml of *P. pentosaceus* TISTR 536 during 2 days of fermentation in NMB with and without nitrite (Ni) and fresh garlic (G)

sample	activity (AU/ml)/hour								
	0	6	12	18	24	30	36	42	48
NMB + 536	0	100	1,600	6,400	25,600	51,200	12,800	12,800	12,800
NMB + Ni,G,536	0	100	400	1,600	12,800	51,200	12,800	12,800	12,800

Interactive effect of bacteriocin-producing and non-bacteriocin-producing LAB, nitrite and fresh garlic on the growth of *S. anatum* in the model broth

Study of interactive effect of bacteriocin-producing strain of TISTR 536, nitrite and fresh garlic on the growth of *S. anatum* compared to nonbacteriocin-producing strain of JCM 5890 was also performed in NMB under the same condition as Nham production. The results (Fig. 3) showed that NMB with 125 ppm nitrite and 5% fresh garlic could retard the growth of *S. anatum* during the early stage of fermentation. When compared to the control broth with the same amount of nitrite and garlic and the same model broth with nonbacteriocin-producing strain of JCM 5890, use of bacteriocin-producer as starter culture exhibited a better result in rapid decreasing the cell of *S. anatum* respectively. This can be explained that during the early stage of NMB fermentation the synergistic effect of nitrite and disrupted fresh garlic could retard the growth of *S. anatum* [6]. The added strain of TISTR 536 in NMB could also rapidly produce numerous of lactic acid during fermentation, which led to a rapid decrease in pH and compromised the outer membrane of this gram-negative pathogen as described by Stevens *et al.* [9] and Kalchayanand *et al* [10]. After 12 hour of fermentation, an antagonistic produced of TISTR 536 in NMB plus nitrite and fresh garlic (Table 1) could exert synergistic effect on the growth of sublethally stressed cells of *S. anatum* most efficiency [9, 10], which led to a rapid decrease of *S. anatum*.

Conclusions

The study implied the advantage of using selected bacteriocin-producing *P. pentosaceus* TISTR 536 from Nham as starter for Nham production. This bacteriocin-producer strain in combination with 125 ppm of nitrite and 5 % of disrupted fresh garlic exerted a better result to control *S. anatum* than the nonbacteriocin-producing strain of JCM 5890. Thus, TISTR 536 is the potential strain to use as a starter culture for Nham production in order to obtain a salmonella-free Nham product.

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