Synergistic activity of bacteriocin-producing lactic acid bacteria as starter culture and fresh garlic against *Salmonella* Typhimurium in Nham model broth, Thai fermented meat product

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***Abstract*- Synergistic activity of bacteriocin-producing lactic acid bacteria (LAB), *Lactobacillus salivarius* K4, as probiotic starter culture and fresh garlic against *Salmonella* Typhimurium in Nham Model broth (NMB) was determined. Study of fresh garlic effect in NMB on the growth of *Lb. salivarius* K4 and lactic acid percentage produce during fermentation was evaluated. Moreover, the synergistic inhibition of *S.* Typhimurium by *Lb. salivarius* K4 and fresh garlic in NMB was also investigated. The result showed that *Lb. salivarius* K4 could grow in both NMB treatments, but the number of *Lb. salivarius* K4 in NMB with fresh garlic was lower than *Lb. salivarius* K4 in NMB without fresh garlic after 1, 2 and 3 day fermentation. Whereas, synergistic effect study on *S.* Typhimurium revealed that only fresh garlic or *Lb. salivarius* K4 starter culture treatment had no effect to inhibit *S.* Typhimurium in the first day of fermentation. However, on day 2 and 3 of fermentation *S.* Typhimurium grew slowly in NMB. Interestingly, adding of fresh garlic and *Lb. salivarius* K4 in NMB was completely inhibit *S.* Typhimurium on the first day of fermentation.**

Index Terms— **Synergistic, bacteriocin-producing lactic acid bacteria, Nham model broth**

I. INTRODUCTION

Nham is a popular Thai-style fermented meat product that mainly composed of lean meat (pork or beef), boiled rinds, cooked rice, garlic, salt, sugar, pepper, chilli and sodium nitrite (Valyasevi & Rolle, 2002). Nham is normally consumed without cooking and consider being a ready-to-eat after 3-4 days of fermentation process (Swetwiwathana, Lotong, Nakayama & Sonomoto, 2007). It was found that Nham sold in retail markets has been found to be contaminated with pathogenic bacteria such as *Salmonella* spp., *Staphylococcus* spp., and *Listeria monocytogenes* (Chokesajjawatee et al. 2009). Pathogens contamination can be reduced by the use of appropriate starter culture to achieve an acidic pH of ≤ 4.6 during fermentation (Luxananil et al. 2009). Leroy & De vuyst (2005) reported that bacteriocin-producing lactic acid bacteria may apply as novel functional starter cultures for sausage fermentation. Swetwiwathana et al. (2007) studied of the interactive effects of the bacteriocin-producing strain of *Pediococcus pentosaceus* TISTR 536 as starter culture and fresh garlic on the growth of *S.* Anatum. The result found that an antagonist produced by TIST 536 and fresh garlic could exert synergistic effects on the growth of *S.* Anatum most efficiency and led to a rapid decreased of *S.* Anatum.

Lactobacillus salivarius K4 isolated from chicken intestine and it produced at least 2 bacteriocins, Salivaricin B and a novel salvicin K. Crude bacteiocins produced by this strain exhibited antibacterial activities against *Lb. sakei* subsp. *sakei* JCM 1157^T, *Leu. mesenteroides* subsp. *mesenteroides* JCM 6124^T , *B. coagulans* JCM 2257^T, *E. faecalis* JCM 5803^T , *L. innocua* ATCC 33090^T and *Br. campestris* NBRC 11547 ^{T} (Pilasombut, 2006; Pilasombut et al. 2005). This strain could survive in ox-bile concentration up to 12%. However, in the presence of 3% chicken bile revealed slightly decreased in cell number, but no survival was found in bile salts. This strain survived in gastrointestinal tract model at pH 3, 4 and 7 (Pilasombut, Ngamyeesoon & Swetwiwathana, 2010). Therefore, the objective of this research was to study the synergistic activity of *Lactobacillus salivarius* K4 as probiotic starter culture and fresh garlic against *Salmonella* Typhimurium in NMB.

II. MATERIALS AND METHODS

A. Microorganisms

Bacteriocin-producing LAB*, Lb. salivarius* K4, was obtained from Meat Microbial Laboratory, King Mongkut's Institute of Technology Ladkrabang, Bangkok, Thailand. *S.* Typhimurium TISTR 292 was obtained from Thailand Institute of Scientific and Technological Research. *Lb. salivarius* K4 was propagated in MRS broth (de Man Rogosa and Sharpe; Merck, Germany) at 37˚C for 16 hr under anaerobic condition for optimum growth as previous studied (Pilasombut 2006). Tryptic soy broth (Merck, Germany) with 0.6% Yeast extract (Merck, Germany) was used for cultivation of *S.* Typhimurium TISTR 292.

B. Preparation of fresh sterile garlic for NMB

Fresh garlic were gently peeled off, then washed with sterile distilled water and soaked in 70% ethanol for 30 min. Subsequently, garlic was washed thoroughly again with sterile distilled water. Aseptic garlic was cut into small pieces and then applied into the sterile NMB (Swetwiwathana, Leutz, Lotong & Fischer, 1999; Swetwiwathana et al. 2007).

C. Nham Model broth (NMB)

The Nham model broth (NMB, Swetwiwathana, Leutz, Lotong, & Fischer, 1999), which simulated the condition of Nham production (aw 0.970, pH 6.3, microaerophilic condition with paraffin oil, 100 ppm/ml of filter-sterilized sodium nitrite added and 5% sterilized fresh garlic), was used as a model broth. Bacteriocin-producing LAB strain (*Lb. salivarius* K4) was used at level of 10^6cfu/ml as starter culture. *S.* Typhimurium TISTR 292 (10^2 cfu/ml) was inoculated in NMB. Study of fresh garlic effect on the growth of *Lb. salivarius* K4 in NMB was designed into 2 treatments; 1) *Lb. salivarius* K4 in NMB; 2) *Lb. salivarius* K4 and sterilized fresh garlic in NMB. For studying the synergistic effect of bacteriocin-producing LAB strain (*Lb. salivarius* K4) and sterilized fresh garlic on the growth of *S.* Typhimurium TISTR 292, the experiment composed of 4 groups as the following; 1) NMB inoculated with *S.* Typhimurium TISTR 292, 2) NMB inoculated with *Lb. salivarius* K4 and *S.* Typhimurium TISTR 292, 3) NMB inoculated with *S.* Typhimurium TISTR 292 and sterilized fresh garlic and 4) NMB inoculated with *Lb. salivarius* K4, *S.* Typhimurium TISTR 292 and sterilized fresh garlic. The samples were left to ferment at 30 °C after *Lb. salivarius* K4 and *S.* Typhimurium TISTR 292 inoculation. The pH, *Lb. salivarius* K4 growth and number of *S.* Typhimurium TISTR 292 were determined at 0, 1, 2 and 3 day fermentation (Swetwiwathana et al., 1999; Swetwiwathana et al., 2007). Moreover, pH value of NMB was measured by pH meter model EUTECH PC 510 (Singapore). *Lb. salivarius* K4 and *S.* Typhimurium TISTR 292 colonies were counted by spread plate on MRS and TSB agar, respectively and expressed as colony forming unit (cfu/ml). In addition, *S.* Typhimurium was confirmed by XLD agar (Merck, Germany).

III. RESULTS AND DISCUSSION

1. Effect of fresh garlic on the growth of *Lb. salivarius* K4 in NMB

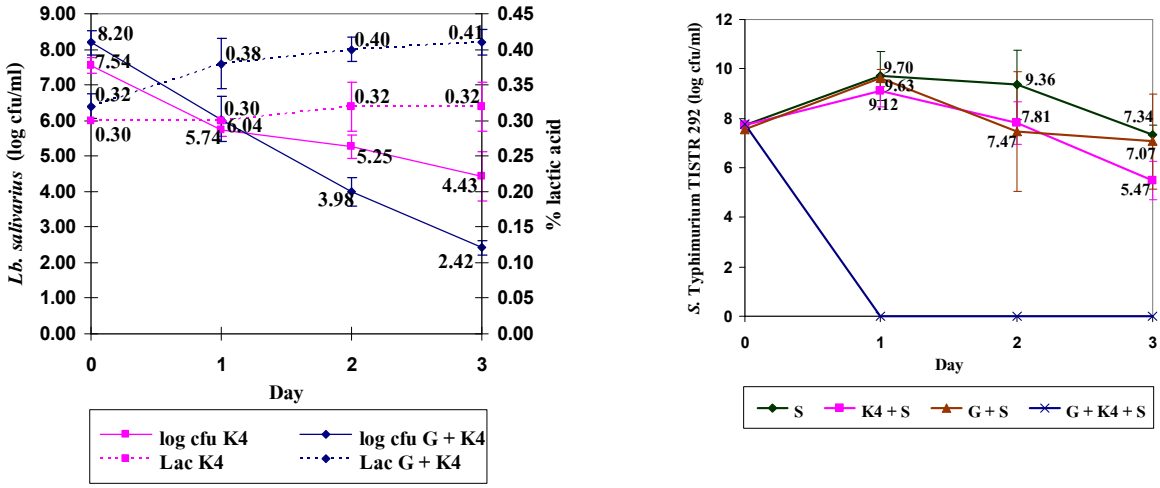
The growth of *Lb. salivarius* K4 in NMB with and without fresh garlic was studied. The result observed that *Lb. salivarius* K4 could grow in both NMB treatments, but the number of *Lb. salivarius* K4 in NMB with fresh garlic was lower than *Lb. salivarius* K4 in NMB without fresh garlic after 1, 2 and 3 day fermentation. Numbers of *Lb. salivarius* K4 in NMB with garlic after fermentation 1, 2 and 3 days was 6.04, 3.98 and 2.42 log cfu/ml, respectively when compared with initial number at 0 day (8.20 log cfu/ml). However, number of *Lb. salivarius* K4 in NMB without fresh garlic was higher than another after fermentation 2 and 3 days. The percentage of lactic acid produced by *Lb. salivarius* K4 in NMB with and without fresh garlic was also evaluated. The results displayed that lactic acid percentage in NMB with fresh garlic was higher than NMB without fresh garlic. This contradicted with number of *Lb. salivarius* K4 reported earlier. Since, it was noticed that the initial number of *Lb. salivarius* K4 in NMB with fresh garlic at day 0 and day 1 was higher than *Lb. salivarius* K4 in NMB without fresh garlic. Therefore this could cause *Lb. salivarius* K4 in NMB with fresh garlic produced more lactic acid in the first day of fermentation and lactic acid still exist in NMB until day 3 (Figure 1 (a)).

2. Synergistic activity of *Lb. salivarius* K4 as starter culture and fresh garlic against *Salmonella* Typhimurium in NMB

The study of synergistic activity of *Lb. salivarius* K4 as starter culture and 5% fresh garlic against *S.* Typhimurium in NMB was performed. The results in Figure 1 (b) revealed that only fresh garlic or *Lb. salivarius* K4 starter culture

treatment had no effect to inhibit *S.* Typhimurium TISTR 292 in the first day of fermentation. However, *S.* Typhimurium TISTR 292 could slowly grew in NMB in day 2 and 3 of fermentation. Interestingly, adding of fresh garlic and *Lb. salivarius* K4 was completely inhibit *S.* Typhimurium TISTR 292 after the first day of fermentation in NMB. This can be explained that allicin (S-allyl-Lcysteine-S-oxide) from garlic is known as the principal antimicrobial component to exhibit a wide spectrum of antibacterial activity against Gram-negative and Gram-positive bacteria including species of *Escherichia, Salmonella, Staphylococcus, Streptococcus, Klebsiella, Proteus, Bacillus, Clostridium and* also acid-fast bacteria (Ankri & Mirelman, 1999). This result was similar to Swetwiwathana et al. (1999) as previously reported. He found that the use of various starter cultures (*L. curvatus, L. sakei and P. acidilactici*) combined with 5% fresh garlic had more effect to inhibit *S.* Anatum in NMB during the first day of fermentation. He also reported that sodium nitrite at 125 ppm decreased the number of *S.* Anatum when compared to broth without curing salts. It appeared that despite pH value of NMB with fresh garlic and *Lb. salivarius* K4 was higher (4.47) than other treatments, *S.* Typhimurium TISTR 292 was completely inhibited (Table 1). This could support the synergistic effect of garlic, bactericin and acid which produced by *Lb. salivarius* K4. Many publications reported the synergistic activity of bacteriocin and garlic against pathogenic bacteria (Singh, Falahee & Adams, 2001; Kim, Choi, Bajpai, & Kang, 2008). In addition, Swetwiwathana et al. (2007) studied the synergistic effect of lowering pH using lactic acid and the addition of crude pediocin PA-1 on *S.* Anatum in trypticase soy broth. The result revealed that most of pathogen becomes sublethally injured. The same results were confirmed in NMB adding 5% garlic and pediocin producer as starter.

Therefore, this study indicated that *Lb. salivarius* K4 could be probiotic starter culture for Nham and possible to make a safe product for consumer.



(a) (b)

Figure 1 (a) Effect of fresh garlic on the growth of *Lb. salivarius* K4 in NMB; log cfu K4 = number of *Lb. salivarius* K4 in NMB; log cfu G+K4 = number of *Lb. salivarius* K4 when added fresh garlic in NMB; Lac K4 = % lactic acid production produced by *Lb. salivarius* K4; Lac G+K4 = % lactic acid production produced by *Lb. salivarius* K4 when added fresh garlic in NMB

(b) Inhibitory effect of fresh garlic and *Lb. salivarius* K4 on survival of *S.* Typhimurium TISTR 292 in NMB

S = NMB inoculated with *S.* Typhimurium TISTR 292; K4+S = NMB with 10^6 cfu/ml *Lb. salivarius* K4 and *S.* Typhimurium TISTR 292; G+S = NMB with fresh garlic and *S.* Typhimurium TISTR 292; G+K4+S = NMB with fresh garlic, *Lb. salivarius* K4 and *S.* Typhimurium TISTR 292

Table 1 pH value of NMB

NMB	pH			
	Day 0	Day 1	Day 2	day 3
Salmonella (S)	5.86±0.08	4.31±0.10	4.12±0.05	4.07±0.10
K4 + S	5.65±0.06	4.34±0.07	4.17±0.03	4.08±0.10
Garlic (G) + S	5.70±0.07	5.04±0.67	4.84±0.74	4.32±0.06
G+ K4 + S	5.38±0.33	4.71±0.27	4.65±0.24	4.47±0.14

S = NMB inoculated with *S.* Typhimurium TISTR 292

K4+S = NMB with 10^6 cfu/ml *Lb. salivarius* K4 and *S.* Typhimurium TISTR 292

G+S = NMB with fresh garlic and *S.* Typhimurium TISTR 292

G+K4+S = NMB with fresh garlic, *Lb. salivarius* K4 and *S.* Typhimurium TISTR 292

IV. CONCLUSION

These results indicated that fresh garlic and *Lb. salivarius* K4 starter culture act synergistically against *S.* Typhimurium in Nham Model Broth (NMB). Therefore, *Lb. salivarius* K4 and garlic can be use for starter culture in Nkam, a Thai fermented meat product.

ACKNOWLEDGEMENT

This research is partially supported by Meat Technology Research Network Center of King Mungkut's Institute of Technology Ladkrabang on corresponding between King Mongkut's Institute of Technology Ladkrabang and the Thailand Research Fund.

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