EFFECT OF CHITOSAN ON LACTIC ACID BACTERIA IN NHAM, A TRADITIONAL THAI FERMENTED MEAT, MODEL BROTH SYSTEM

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Abstract - The effect of 0, 100, 500, 1000, 2000 and 3000 ppm of Chitosan (CS) on lactic acid bacteria (LAB) associated in Nham was studied using the spot-on-lawn method. CS concentrations from 500 to 3000 ppm exhibited an antimicrobial effect on *Lb. plantarum* ATCC 14917, while for *P. pentosaceus* TISTR 536 1000 to 3000 ppm of CS was required for inhibition. In this study, a Nham model broth system was used to confirm the effect of CS at concentrations of 100, 500, 1000, 5000 and 10000 ppm on each LAB strain. CS at 100 ppm could inhibit the growth of *Lb. plantarum* ATCC 14917, whereas, *P. pentosaceus* TISTR 536 was inhibited at 500 ppm of CS. At higher concentrations of CS (500 to 10000 ppm) cell numbers of *Lb. plantarum* ATCC 14917 were reduced within 48 h, while *P. pentosaceus* TISTR 536 was not detectable in the NMB within 48 h at CS concentration of 1000 to 10000 ppm. The study showed that *P. pentosaceus* TISTR 536 exhibited a higher resistance to Chitosan than *L. plantarum* ATCC 14917.

Key Words – chitosan, *Lactobacillus plantarum, Pediococcus pentosaceus,* Nham, traditional Thai fermented meat

I. INTRODUCTION

Chitosan (CS) is a non-toxic antimicrobial that also possesses functional properties including intestinal lipid binding and serum cholesterol lowering effects [1, 2], water binding [3], antioxidative and preservative effects in muscle foods [4] and emulsifying capacity [5]. For these reasons, CS is of interest to the food industry. Chitosan, a polysaccharide comprising copolymers of glucosamine and N-acetyl-glucosamine, is obtained after alkaline deacetylation of the chitin derived from the exoskeletons of crustaceans and arthropods. Antimicrobial activity is the result of disruption of the bacterial cell membrane, with the release of cellular contents [6]. There are many reports of studies of the inhibitory effect of CS on various gram negative and gram positive bacteria including *Salmonella* spp. and *Staphylococcus aureus* [7, 8]. The main objective of our previous study was to investigate whether CS (concentration of 100 500 and 1000 ppm) has an antimicrobial effect on some pathogens (*Salmonella* Anatum, *Salm.* Derby and *Staphylococcus aureus*) which are associated with Nham (a popular fermented sausage in Thailand, mainly composed of lean pork, sliced cooked pork rind, cooked rice, garlic and salts) during fermentation. The results showed that higher concentrations of chitosan (500 and 1000 ppm) in NMB were more effective at inhibiting pathogens studied than 100 ppm [9].

In this study, the effect of CS on two lactic acid bacteria (LAB), *Lactobacillus plantarum*, which mostly associated with various traditional Thai fermented meat products [10], and *Pediococcus pentosaceus* TISTR 536, which is a pediocin PA-1 producing strain isolated from Nham [11] was studied using the spot-on-lawn method and a Nham Model Broth (NMB) system

II. MATERIALS AND METHODS

Preparation of chitosan solution:
Chitosan (85% Degree of deacetylation, A.N. LAB Thailand) at concentrations of 0, 100, 500, 1000, 2000 and 3000 ppm in 1% (v/v) lactic acid were prepared and adjusted to pH 6.0 with 1 N NaOH. Chitosan solutions were sterilized at 121°C for 15 min [12].

Preparation of bacteria:
To obtain lactic acid bacteria (LAB) inocula for the experiments, *Pediococcus pentosaceus* TISTR 536 [11] and *Lactobacillus plantarum* ATTC 14917 from deep tube MRS agar stock cultures

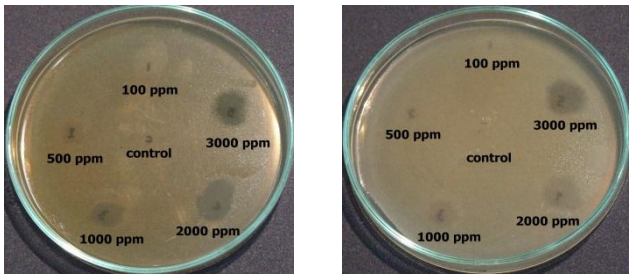
were subcultured twice in MRS broth which was incubated for 18 to 20 h at 30°C.

Determination of antimicrobial activity of CS using spot on lawn assay:
The effect of each CS concentration on each LAB strain was determined using the spot-on-lawn method [11]. Ten µl of overnight culture of each LAB were transferred to 5 mL of molten MRS with 1% agar and poured over an MRS agar plate. Ten µL of CS at each concentration was spotted onto the surface of the plates. The antibacterial activity was evaluated after incubation for 24 h at 30°C by measurement of the zones of inhibition.

Determination of antimicrobial activity of CS in NM:
CS at concentrations of 0, 100, 500, 1000, 5000 and 10000 ppm were prepared in NMB [13] and the pH adjusted to 6.0. After sterilization at 121°C for 15 min, bacteria from an overnight culture of each LAB were inoculated into the NMB with a final concentration of bacteria in the NMB of *ca*. 10^6 cfu/mL. All NMB samples were incubated at 30 to 32°C anaerobically for 48 h. Anaerobic conditions were established by pouring sterile paraffin oil over the NMB. The survival of the LAB in NMB was determined every 6 h by the spread plating. Samples were plated onto MRS agar and incubated at 30°C for 48 h in a candle jar.

III. RESULTS AND DISCUSSION

Effect of CS on LAB:
The effect of CS on *Lactobacillus plantarum* ATCC 14917 and *Pediococcus pentosaceus* at concentrations of 100, 500, 1000, 2000 and 3000 ppm was studied. The results (Fig. 1) showed that CS concentrations from 500 to 3000 ppm exhibited an antimicrobial effect on *Lb. plantarum* ATCC 14917 (a). For *P. pentosaceus* TISTR 536 concentrations from 1000 to 3000 ppm (b) were required for inhibition. The study showed that *P. pentosaceus* TISTR 536 could tolerate higher concentrations of CS than *Lb. plantarum* ATCC 14917. These results are in agreement with earlier reports of Darmadji and Izumimoto [4] and No et al. [7].



(a) (b)

Figure 1. Effect of CS at different concentrations on (a) *Lb. plantarum* ATCC 14917 and (b) *P. pentosaceus* TISTR 536

Effect of CS on LAB in NMB:
To confirm the results of the antimicrobial effect of CS on LAB, the effect of each of the CS concentrations (100, 500, 1000, 5000 and 10000 ppm) in NMB was measured. *P. pentosaceus* TISTR 536 and *Lb. plantarum* ATCC 14917 were introduced to NMB at final concentrations of 10^6 cfu/mL and their growth in the presence of CS was compared to samples in which no antimicrobial had been added (Fig. 2). Results showed that both LAB could grow in NMB. When CS was added at a concentration of 100 ppm, there was some inhibitory effect on *Lb. plantarum* ATCC 14917 during 48 h of NMB fermentation, but this CS concentration showed no effect on *P. pentosaceus* TISTR 536. When the CS concentration was 500 ppm and above, *Lb. plantarum* ATCC 14917 did not grow during 48 h of NMB fermentation. At concentrations of CS from 1000, 5000 and 10000 ppm, *Lb. plantarum* ATCC 14917 could not be recovered from NMB within 24, 12 and 12 h, respectively. CS concentration from 500 ppm reduced the cell numbers of *P. pentosaceus* TISTR 536 from 10^6 cfu/mL to 10^2 cfu/mL within 48 h of NMB fermentation. When the CS concentration was 1000, 5000 and 10000 ppm, *P. pentosaceus* TISTR 536 could not be recovered from NMB within 48, 48 and 36 h, respectively. These observations confirm results from the spot-on-lawn assay. This study suggests that *P. pentosaceus* TISTR 536 could be used as a starter culture for Nham production and that CS at a concentration of at least 100 ppm could be added to control growth of bacterial pathogens without inhibiting *P. pentosaceus* TISTR 536.

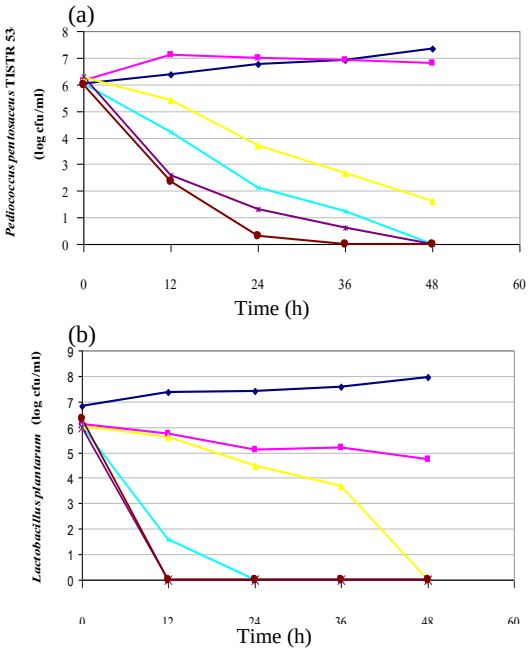


Figure 2. Effect of chitosan on the growth of *P. pentosaceus* TISTR 536 (a) and *Lb. plantarum* ATCC 14917 (b). Control without chitosan (♦), 100 ppm (■), 500 ppm (▲), 1000 ppm (x), 5000 ppm (*) and 10,000 ppm (●)

IV. CONCLUSION

The results of this work show that *P. pentosaceus* TISTR 536 could potentially be used as a starter culture in the production of Nham and that chitosan could be included to enhance the microbiological quality and safety of this traditional Thai fermented meat product without inhibiting the *P. pentosaceus* TISTR 536 starter culture. The synergistic effects of chitosan and other ingredients, including the effect of pediocin PA-1 produced by *P. pentosaceus* TISTR 536, during Nham production on all strains of Nham-associated pathogens are under investigation.

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REFERENCES

1. Maezaki, Y., Tsuji, K., Nakagawa, Y., Kawai, Y., Akimoto, M., & Tsugita, T. (1993). Hypocholesterolemic effect of chitosan in adult males. Bioscience, Biotechnology and Biochemistry 57: 1439–1444.
2. Razdan, A., & Pettersson, D. (1994). Effect of chitin and chitosan on nutrient digestibility and plasma lipid concentrations in broiler chickens. British Journal of Nutrition 72: 277–288.
3. Knorr, D. (1982). Functional properties of chitin and chitosan. Journal of Food Science 47: 593–595.
4. Darmadji, P., & Izumimoto, M. (1994). Effect of chitosan in meat preservation. Meat Science 38: 243–254.
5. Lee, S. H. (1996). Effect of chitosan on emulsifying capacity of egg yolk. Journal of Korean Society of Food Nutrition (Taiwan) 23: 608–616.
6. Hui, L., Yumin, D., Xiaohui, W., & Sun, L. 2004. Chitosan kills bacteria through cell membrane damage. International Journal of Food Microbiology 95: 147-155.
7. No, H.K., Park, N.Y., Lee, S.H., & Meyers, S.P. (2002). Antibacterial activity of chitosans and chitosan oligomers with different molecular weights. International Journal of Food Microbiology 74: 65-67.
8. Fujimoto, T., Tsuchiya, Y., Terao, M., Nakamura, K., & Yamamoto, M. (2006). Antibacterial effect of chitosan solution against *Legionella pneumophila, Escherichia coli, and Staphylococcus aureus*. International Journal of Food Microbiology 112: 96-101.
9. Saisawart, P., Jindaprasert, A., Krusong, W., & Swetwiwathana, A. 2010. Effect of chitosan on associated bacterial pathogens in Nham (traditional Thai fermented meat) model broth. The 56th International Congress of Meat Science and Technology (ICoMST) Proceedings (in CD). August 15-20, 2010 Jeju, South Korea.
10. Swetwiwathana, A., Zendo, T., Nakayama, J., & Sonomoto, K. 2008. A Novel Pediocin-like Produced by *Lactobacillus plantarum* KMITL-QU 54 from Traditional Thai Fermented Meat-rice Sausage (Sai-krog Isan). The 54th International Congress of Meat Science and Technology (ICoMST) Proceedings (in CD). August 10-15, 2008 Capetown, South Africa.

11. Swetwiwathana, A. 2005. Microbiological quality enhancement of Thai fermented meat product (Nham) using Nham-associated pediocin-producing lactic acid bacteria (*Pediococcus pentosaceus* TISTR 536). Ph.D. Thesis. Laboratory of Microbial Technology, Division of Microbial Science and Technology, Department of Bioscience and Biotechnology, Graduate School of Bioresource and Bioenvironmental Sciences, Kyushu University.

12. Jongsareejit, B., Nakplaipan, P., & Jirachanchai, S. (2004). Chitosan : study of antimicrobial activity in food. Journal of Science (Thailand) 58: 88-95.

13. Swetwiwathana, A., Leutz, U., Lotong, N., & Fischer A. (1999). Controlling the growth of *Salmonella anatum* in nham. Effect of meat starter culture, nitrate, nitrite and garlic. Fleischwirtschaft 79:124-128.