In vitro studied of Lactic acid bacteria as probiotic starter for fermented meat product

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Abstract— **Lactic acid bacteria (LAB) named "Sb 2 isolate", which produced bacteriocin and acted as probiotic, was further studied *in vitro* for 3 aspects. The tolerance in 100 ppm sodium nitrite in Nham model for 3 days, survival of the isolate in gastrointestinal tract model and synergistic activity of lactic acid and bacteriocin against *Salmonalla* Anatum were determined. The results showed that isolate Sb 2 could survive in Nham model added sodium nitrite and survival number was closely in model with and without sodium nitrite. The survival of Sb 2 was 6.38 log cfu/ml in the stomach model at pH 3 for 180 mins, but no isolate was found in the intestinal model. However, at pH 4 for 180 mins, the survival of Sb 2 was 6.67 and 5.52 log cfu/ml in stomach and intestinal model respectively. However, it was completely inhibited in the presence of gastric juice at pH 2. In addition, bacteriocin displayed synergistic activity with lactic acid against *S.* Anatum. Therefore, isolate Sb 2 could be used as a probiotic starter culture in fermented meat product.**

Keywords— **probiotic, starter, fermented meat product**

I. INTRODUCTION

Lactic acid bacteria have long been use as starter cultures in the production of fermented dry sausages and other meat-derived commodities. These cultures are generally designed to meet food safety, shelf-life, technological effectiveness and economic feasibility criteria. In addition, functional starters could protect consumers from harmful bacteria by either rapid acidification or the production of antimicrobials (bacteriocins) [1]. [2] reported that bacteriocin-producting lactic acid bacteria may apply as novel functional starter cultures for sausage fermentation. In addition, specially-selected cultures may also provide for probiotic benefits [1]. The probiotic concept has been defined as a live microorganisms that when administered in adequate amounts confer a health benefit on the host [3]. Lactic acid bacteria are regarded as a major group of probiotic bacteria [4]. The important property of probiotic is survival in gastric juice, tolerance to low pH and produce antimicrobial substance [1].

LAB Sb 2 isolated from gastrointestinal tract of seabass (*Lates calcarifer*). This strain was able to produce bacteriocin, which inhibit several bacteria including *Staphylococcus aureus*, *Listeria innocua*, *Enterococcus feacalis*, *Leuconostoc mesenteroides* and *Pseudomonas fluorescens* ect. Moreover, this strain has been shown probiotic properties as it was able to grow and produce bacteriocin when culture in pH 3-10, NaCl 1-5% and survive in gastrointestinal tract model [5,6,7]. Therefore, further studies of Lactic acid bacteria Sb 2 properties as probiotic starter culture were demonstrated.

MATERIALS AND METHODS

A. Microorganisms

Bacteriocin-producing LAB Sb 2 and *S.* Anatum, were obtained from Meat Microbiology Laboratory, King Mongkut's Institute of Technology Ladkrabang, Bangkok, Thailand. LAB Sb 2 was propagated in MRS broth (de Man Rogosa and Sharpe; Merck, Germany) at 30 ˚C for 16 hr under anaerobic condition for optimum growth as previous studied [7]. Tryptic soy broth (Merck, Germany) with 0.6% Yeast extract (Merck, Germany) was used for cultivation of *S.* Anatum.

B. Effect of nitrite on LAB Sb 2

The Nham model broth (NMB) [8], 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), was used as a model broth. LAB Sb 2 of 10^6 cfu/ml was added in NMB and incubated at 30 °C. For control treatment 100 ppm/ml of filter-sterilized sodium nitrite was omitted in NMB. The numbers of LAB Sb 2 survival were determined by total plate count.

C. Determination of artificial gastric and intestinal fluids tolerance

Simulated gastric digestion was tested essentially as described in [9]. LAB Sb 2 was inoculated in 100 ml of MRS broth at 2% (v/v) and incubated at 30˚C for 16 hr. After washing in sterile saline solution (0.9% NaCl) and centrifugation, the cell suspension was added to 100 ml of artificial gastric juice with the following composition: NaCl 125 mM, KCl 7 mM, $NaHCO_3$ 45 mM, and pepsin (Fluka, Switzerland) 3 g /L. The final pH was adjusted with HCl solution to pH 2, 3, 4, and 7. The bacterial suspension was agitated at 200 rpm/min to simulate peristalsis. Aliquots were taken for enumeration of viable at 0, 30, 60, 90 and 180 min by spread plate technique with MRS agar containing 0.5% $CaCO_3$. Simulated intestinal fluid was prepared by suspending the cells (after 180 min of gastric digestion) in 0.1% (w/v) pancreatin (Fluka, Switzerland) and 0.15 % (w/v) bile salts (Sigma, New Zealand) in water and adjusted it to pH 8.0 with 1 N NaOH solution. The suspension was incubated as described above and samples for total viable counts were taken at 0, 30, 60, 90 and 180 min using spread plate technique with MRS agar containing 0.5% $CaCO_3$. The experiment was performed in triplicate and mean were calculated.

D. Synergistic effect of crude bacteriocin and lactic acid on survival of S. Anatum

Tryptic soy broth (TSB) was prepared and adjusted to pH 4.5 and 5.0 with lactic acid (90%). Sterile crude bacteriocin (2%) isolated from LAB Sb 2 was added into both pH. Culture of *S.* Anatum was transferred to different pH of TSB and then incubated at 37 °C for 30 hr. Survival number of *S.* Anatum was investigated every 6 hr, by pour plate technique with Tripticase Soy Agar (TSA, Merck, German) [9, 10].

RESULTS AND DISCUSSION

A. Study on effect of nitrite on LAB Sb 2

The study showed that LAB Sb 2 could survive in Nham model broth with 100 ppm/ml sodium nitrite through 3 days of fermentation. The number of isolate Sb 2 in Nham model added sodium nitrite was closely in model without sodium nitrite. (Fig. 1). [11] reported that nitrite was required in sausage fermentation technology as curing agent for microbe stability and color formation. However, nitrite was a hurdle against the growth of *Salmonella* sp., *Staphylococcus aureus* and *Clostridium* sp. [12]. Since sodium nitrite is used as a main ingredient in fermented meat products, survival of LAB Sb 2 gives promising sign as starter culture.

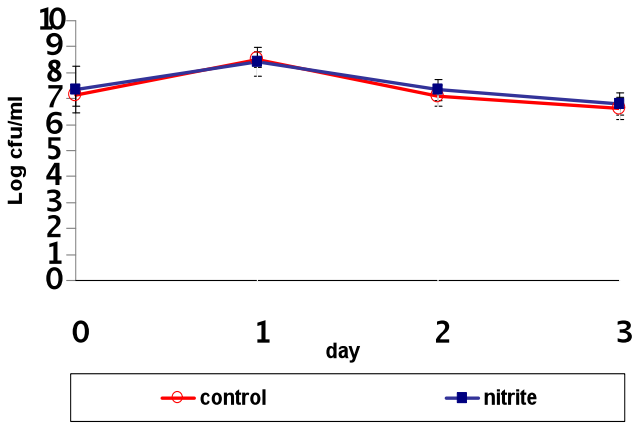

Fig. 1 Effect of sodium nitrite in Nham model broth on LAB Sb 2 survival

B. Determination of artificial gastric and intestinal fluids tolerance

The tolerance of LAB Sb 2 in artificial gastric and intestinal model at pH 2, 3, 4, and 7 for 180 mim each showed that there was no survival of LAB Sb2 at pH 2. Although, in the stomach model at pH 3, 4, and 7 survival number were 6.38, 6.67 and 6.83 log cfu/ml

(180 min) respectively, whereas initial number was 7.56 log cfu/ml. While, the survival figures in intestinal model were 0, 5.52 and 6.42 log cfu/ml respectively, (Table 1). One of the most important criteria for selecting lactic acid bacteria as probiotic was survival of microorganisms in the gastrointestinal conditions [9]. These indicated that LAB Sb2 had probiotic property to withstand artificial gastric and intestinal fluids.

C. Synergistic effect of crude bacteriocin and lactic acid on survival of S. Anatum

The effect of crude bacteriocin produced by LAB Sb 2 and lactic acid on *S.* Anatum was investigated. The result found that *S.* Anatum number decreased coincident with longer incubation period and lower pH values (Fig. 2, 3). The *S.* Anatum number after 30hr incubation was 2.48 and 5.57 log cfu/ml at pH 4.5 and 5.0 respectively. Whereas, growth figure of *S.* Anatum gradually increased with longer incubation time in treatment without crude bacteriocin. Therefore, crude bacteriocin and lactic acid had synergistic effect on survival of *S.* Anatum.

II. CONCLUSIONS

LAB Sb 2 has a good probiotic properties as it could survive in Nham model with sodium nitrite and artificial gastric and intestinal fluids. In addition, there was synergistic effect of crude bacteriocin produced by LAB Sb 2 and lactic acid on survival of *S.* Anatum. As a result of this, consumer could be benefit on safety and healthy food by using LAB Sb 2 as probiotic starter culture in fermented meat product.

Table 1 Effect of artificial gastric and intestinal fluids at pH 2, 3, 4, and 7 for 0 -180 min on survival of LAB Sb 2

pH	Survival number in stomach model (Log CFU/ml) Time (minute)						Survival number in intestinal model (Log CFU/ml) Time (minute)					
	Before	0	30	60	90	180	0	30	60	90	180
2	7.81	7.17	0	0	0	0	0	0	0	0	0
3	7.56	6.98	6.89	6.84	6.66	6.38	6.01	4.11	0	0	0
4	7.56	7.24	7.06	6.90	6.75	6.67	6.18	6.04	5.91	5.70	5.52
7	7.51	7.40	7.26	7.10	6.89	6.83	6.76	6.71	6.58	6.49	6.42

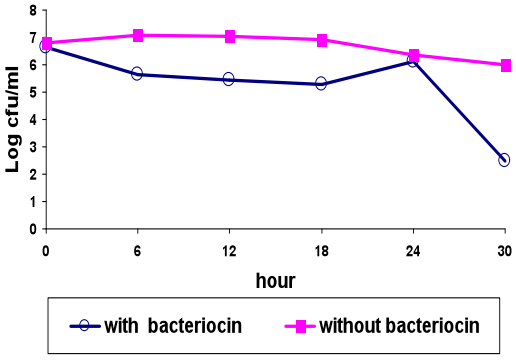

Fig. 2 Synergistic effect of crude bacteriocin and lactic acid on survival of *S.* Anatum at pH 4.5

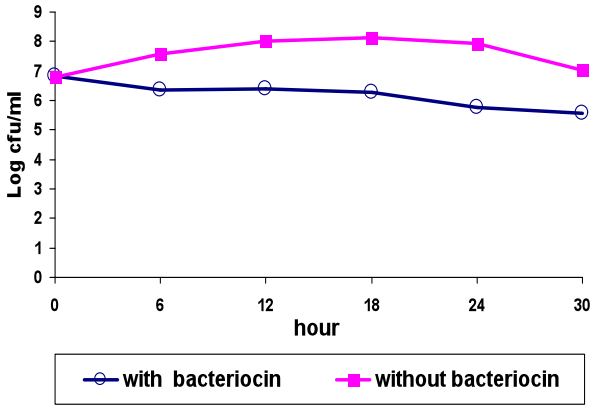

Fig. 3 Synergistic effect of crude bacteriocin and lactic acid on survival of *S.* Anatum at pH 5.0

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REFERENCES

1. Ammor, M.S. and Mayo, B. 2007. Selection criteria for lactic acid bacteria to be used as functional starter cultures in dry sausage production: An update. **Meat Sci**. 76: 138-146**.**
2. Leroy, F. and De Vuyst, L. 2005. Simulation of the effect of sausage ingredients and technology on the functionality of the bacteriocin-producing *Lactobacillus sakei* CTC 494 strain. **Int. J. Food Microbiol**. 100: 141-285.
3. Guarner, F., Perdigon, G,. Corthier, G., Salminen, S., Koletzko, B. and Morelli, L. 2005. Should yogurt cultures be considered probiotic. **British Journal of Nutrition** 93: 783-786.
4. Collins, J.K., Thornton, G. and Sullivan, G.D. 1998. Selection of probiotic strains for human applications. **Int. Dairy J.** 8: 487-490.
5. Pilasombut, K., Sethakul, J. and Swetwiwathana, A. 2010. Probiotic properties of bacteriocin-producing lactic acid bacteria isolated from seabass gastrointestinal tract. **King Mongkut's Agricultural Journal.** 28 (3): 1-8.
6. Rumjuankiat, K., Pilasombut, K., Wangwibulkit, S. and Swetwiwathana, A. 2010. Screening and parcial characterization of bacteriocin from lactic acid bacteria in fish gastrointestinal tract. **KKU Res. J.** 15 (9): 870-877.
7. Rumjuankiat, K. 2010. Isolation and characterization of bacteriocin produced by lactic acid bacteria from fish gastrointestinal tract. M. Sc. Thesis, King Mongkut's Institute of Technology Ladkrabang, Thailand.
8. Swetwiwathana, A., Leutz, U., Lotong, N., and Fischer, A. 1999. Controlling the growth of *Salmonella anatum* in nham; Effect of meat starter cultures, nitrate, nitrite and garlic. ***Fleischwirtschaft***. 79: 124-128.
9. Swetwiwathana, A., Lotong, N., Nakayama, J. and Sonomoto, K. (2007). Effect of Pediocin PA-1 producer (*Pediococcus pentosaceus* TISTR 536) as starter culture, nitrite and garlic on *Salmonella anatum* during Nham fermentation. ***Fleischwirtschaft***. 22: 46-49.
10. Veerawatanayotin, S., Jindaprasert, A., Pilasombut, K., Sethakul, J. and Swetwiwathana, A. 2010. Effect of pediocin PA-1, pH and nitrite on *Salmonella* Anatum and Ratchaburi in simulated Nham (traditional Thai fermented meat sausage) model broth. *In* **56th International Congress of Meat Science and Technology**, Jeju, Korea, August 2010. 5 p.
11. Leroy, F., Verluyten, J. and De Vuyst, L. 2006. Functional meat starter cultures for improved sausage fermentation. **Int. J. Food Microbiol**.106: 270-285.
12. Ryu, J.S. and Lloyd, D. 1995. Cell cytotoxicity of sodium nitrite, sodium nitroprusside and Roussin's black salt against *Trichomonas vaginalis*. **FEMS. Microbiol. Letters.** 130: 183-188.