

THE POTENTIAL USE OF BACTERIOCIN-PRODUCING LACTIC ACID BACTERIA D4 AND P2 AS STARTER CULTURE FOR THAI FERMENTED MEAT PRODUCT

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Abstract - Bacteriocin-producing lactic acid bacteria (LAB) D4 and P2 were isolated from duck and *Pangasianodon hypophthalmus* (striped catfish) intestines, respectively. These strains produced bacteriocin and having probiotic attributes. Therefore, they were further *in vitro* studied for starter culture properties in Thai fermented meat model (Nham). The tolerance of D4 and P2 in 100 ppm sodium nitrite in Nham model broth (NMB) during fermentation for 3 days was carried out. Study of fresh garlic effect in NMB on the growth of D4 and P2 during fermentation was evaluated. In addition, the synergist effect of fresh garlic and LAB strain, D4 or P2, in NMB against *S. Typhimurium* TISTR 292 was also performed. The results showed that both D4 and P2 could survive in NMB added with sodium nitrite. Survival of D4 and P2 in NMB slightly decreased in cell number after being exposed for 2 days. Moreover, adding of fresh garlic and D4 or P2 could inhibit *S. Typhimurium* TISTR 292 on the first day of fermentation. Therefore, bacteriocin-producing LAB, D4 and P2, from animal sources could be used as starter culture in Thai fermented meat products.

Key Words – Lactic acid bacteria, Nham, Starter culture

I. INTRODUCTION

Bacteriocin-producing LAB D4 and P2 were isolated from duck and *Pangasianodon hypophthalmus* (striped catfish) intestines, respectively. Both strains produce bacteriocin and have antibacterial activity against *Lactobacillus sakei* subsp. *sakei* JCM 1157^T, *Lb. sakei* TISTR 890, *Lb. plantarum* ATCC 14917, *Leuconostoc mesenteroides* subsp. *mesenteroides* JCM 6124^T, *Leu. mesenteroides* TISTR 942, *Listeria innocua* ATCC 33090^T, *Brochotrix*

campestris NBRC 11547^T, *Pseudomonas fluorescens* JCM 5693 and *P. fluorescens* TISTR 358, *Lactococcus lactis* subsp. *cremoris* TISTR 1344, *Enterococcus faecalis* JCM 5803^T, *En. faecalis* TISTR 888 and *Bacillus coagulans* JCM 2257. Moreover, bacteriocin of P2 demonstrated more spectrums against *Staphylococcus aureus*. Isolate D4 and P2 also act as probiotic properties. It was found that isolate D4 and P2 could grow in wide pH range 3-10, 0.3-0.9% bile salt and 8% NaCl. The highest antibacterial activity was shown at pH 7-9 and at 1% NaCl. Moreover, both isolates could grow and produced bacteriocin at high concentrations of NaCl (8%) [4]. In this study, the inhibitory activity and probiotic properties of D4 and P2 were investigated for probiotic starter cultures in model of Thai fermented product (Nham).

II. MATERIALS AND METHODS

1. Cell culture propagation

Lactic acid bacteria, isolate D4 and P2, were propagated in MRS broth and MRS adding 1% NaCl, respectively (de Man Rogosa and Sharpe; Merck, Germany) at 30°C for 18 hr. Tryptic soy broth (Merck, Germany) with 0.6% Yeast extract (Merck, Germany) was used for cultivation of *S. Typhimurium* TISTR 292.

2. Effect of sodium nitrite on isolate D4 and P2 in Nham Model broth (NMB)

Nham model broth (NMB) [7], a simulation 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. Isolate D4 or P2 was inoculated in NMB and incubated at 30 °C. For control treatment, 100 ppm/ml of filter sterilized sodium nitrite was omitted in NMB. The number of isolate D4 and P2 survival were determined by total plate count

3. Antimicrobial effect of fresh garlic and its interaction with isolate D4 or P2 against *S. Typhimurium* in NMB

Study of fresh garlic effect on the growth of isolate D4 and P2 in NMB was designed into 2 treatments; 1) isolate D4 or P2 in NMB; 2) Isolate D4 or P2 and sterilized fresh garlic in NMB. For studying the synergistic effect of bacteriocin-producing LAB isolate D4 or P2 and 5% 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 LAB strain (D4 or P2) and *S. Typhimurium* TISTR 292, 3) NMB inoculated with *S. Typhimurium* TISTR 292 and sterilized fresh garlic and 4) NMB inoculated with LAB strain (D4 or P2), *S. Typhimurium* TISTR 292 and sterilized fresh garlic. The samples were left to ferment at 30 °C after LAB strain (D4 or P2) and *S. Typhimurium* TISTR 292 inoculation. The number of *S. Typhimurium* TISTR 292 was determined at 0, 1, 2 and 3 day fermentation [7,8]. Colonies were counted by spread plate on Tryptic Soy Broth (adding 0.6% Yeast-extract; Merck, Germany) agar and expressed log₁₀ colony forming unit (CFU/ml).

III. RESULTS AND DISCUSSION

1. Inhibitory effect of sodium nitrite on isolate D4 and P2

LAB strain, D4 and P2, could survive in NMB adding 100 ppm/ml sodium nitrite through 3 days of fermentation. Both isolates survival in NMB adding sodium nitrite as compared to the control group did not show much difference in cell number (Table 1). However, the number of both isolates decreased after 3 days. Nitrite is a common food additive in fermented meat products for many proposes, such as improve color and texture, inhibiting the growth of food-borne pathogens as well as a potent of antioxidant [9]. In this study, nitrite has small effect on the growth of isolate D4 and P2. This result was similar to Siththigripong *et al.* [6] who studied the effect of sodium nitrite 100 ppm in NMB on bacteriocin - producing LAB Sb2. The isolate Sb2 could survive with closely number in NMB without sodium nitrite.

Table 1 Inhibitory effects of sodium nitrite on survival of isolate D4 and P2

	Day	Number of LAB (log cfu/ml)	
		Control	Sodium nitrite
D4	0	7.12 ± 0.10	7.03 ± 0.28
	1	8.80 ± 0.50	7.43 ± 1.48
	2	7.26 ± 0.27	7.30 ± 1.01
	3	6.29 ± 0.75	6.40 ± 0.70
P2	0	7.12 ± 0.31	7.09 ± 0.16
	1	8.30 ± 0.87	7.84 ± 0.34
	2	6.76 ± 0.32	6.68 ± 0.33
	3	6.38 ± 0.22	5.63 ± 0.34

2. Effect of fresh garlic on the growth of isolate D4 and P2

The survival of isolate D4 and P2 in NMB with and without fresh garlic were conducted. Number of both isolates in NMB with fresh garlic was similar to number of isolate D4 and P2 in NMB without fresh garlic after fermentation 1, 2 and 3 days. However, number of isolate D4 during 3 days fermentation was lower in NMB with garlic compared to NMB without garlic (Table 2).

Table 2 Survival of isolate D4 and P2 in NMB with and without fresh garlic during fermentation for 3 days

NMB	Number of isolate D4 and P2 (log cfu/ml + S.D.)			
	Day0	Day1	Day2	Day3
D4	6.67±1.15	6.23±0.46	5.08±0.67	5.03±0.28
G+D4	6.42±1.06	5.62±0.69	5.46±0.55	4.11±0.96
P2	7.53±0.17	7.35±0.32	6.53±0.53	6.03±0.27
G+P2	7.61±0.05	7.89±0.05	6.47±0.04	6.32±0.11

Note : G + D4 = NMB with fresh garlic and isolate D4

G + P2 = NMB with fresh garlic and isolate P2

3. Synergistic activity of D4 or P2 as starter culture and fresh garlic against *Salmonella Typhimurium* TISTR 292 in NMB

The study of synergistic activity of D4 as starter culture and 5% fresh garlic against *S. Typhimurium* TISTR 292 in NMB was performed. The result in Table 3 presented that the number of *S. Typhimurium* TISTR 292 was not decrease in NMB for up to 3 days, while NMB added fresh garlic or D4 starter culture treatment had no inhibitory effect against *S. Typhimurium* TISTR 292 in the first day of fermentation. However, *S. Typhimurium* TISTR 292 was slow growing in NMB in day 2 and 3 of fermentation. Interestingly, adding of fresh garlic and D4 showed synergistic inhibition activity against *S. Typhimurium* TISTR 292 after the first and second day of fermentation in NMB. This combination completely inhibits *S. Typhimurium* TISTR 292 after day 3. The effect of isolate P2 as starter and 5% fresh garlic was detected to have completely inhibition of *S. Typhimurium* TISTR 292 after the first day of fermentation (Table 3).

Garlic is one of spice which often use in products as a flavor enhancement [5]. It also commonly use in Thai fermented products and has an antibacterial effect against some pathogenic bacteria [2]. Allicin (S-allyl-L-cysteine-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* and *Clostridium* [1]. However, lactic acid bacteria are generally less inhibited by garlic. Therefore, it may contribute to the safety without in refrigerating with fermentation [2]. Our results were supported by Swetwathana *et al.* [7] who found that the use of various starter cultures (*L. curvatus*, *L. sakei* and *P. acidilactici*) combined with 5% fresh garlic had more inhibitory effect to *S. Anatum* in NMB during the first day of fermentation. In addition, Rohani *et al.* [3] found that the combination of garlic oil and nisin showed significant anti-listerial activity. Therefore, this study indicated that bacteriocin-producing LAB D4 and P2 from animal sources could be use for probiotic starter culture in Thai fermented meat product (Nham) and possible to make a safe product for consumer.

Table 3 Inhibitory effect of fresh garlic and D4 or P2 on survival of *S. Typhimurium* TISTR 292 in NMB

NMB	Number of <i>S. Typhimurium</i> TISTR 292 (log cfu/ml ± S.D.)			
	Day0	Day1	Day2	Day3
S	7.27±0.31	8.28±1.18	7.68±0.93	7.34±0.31
D4+S	7.47±0.30	9.32±0.45	6.68±0.34	5.30±1.62
G+S	7.19±0.02	7.79±1.47	6.51±0.27	5.44±1.40
G+D4+S	7.20±0.11	4.31±0.30	2.55±0.26	0±0
S	7.56±0.39	8.51±0.10	8.67±0.43	6.82±0.19
P2+S	7.19±0.24	<10	<10	<10
G+S	7.19±0.09	9.13±0.02	8.71±0.32	7.78±0.0
G+S+P2	7.32±0.23	<10	<10	<10

i) S = NMB inoculated with *S. Typhimurium* TISTR 292; ii) D4 (or P2) + S = NMB with 10⁶ cfu/ml D4 (or P2) + and *S. Typhimurium* TISTR 292; iii) G+S = NMB with fresh garlic and *S. Typhimurium* TISTR 292; iv) G+D4 (or P2) + S = NMB with fresh garlic, D4 (or P2)+ and *S. Typhimurium* TISTR 292

< 10 means less than limit of detection

IV. CONCLUSION

Bacteriocin-producing lactic acid bacteria isolate D4 and P2 could survive in NMB adding 100 ppm/ml sodium nitrite through 3 days of fermentation. Both isolate could grow in NMB with and without fresh garlic. However, number of both isolate in NMB with fresh garlic was higher than number of isolate D4 and P2 in

NMB without fresh garlic after fermentation 3 days. Combination of fresh garlic and isolate D4 or P2 showed synergistic inhibitory against *S. Typhimurium* in NMB. Therefore, it is possible to apply bacteriocin-producing LAB D4 or P2 from animal sources as starter culture in Thai fermented meat products (Nham).

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