IN VITRO INHIBITORY ACTIVITIES OF LACTIC ACID SOLUTION AND CRUDE BACTERIOCIN AGAINST MEAT PATHOGENIC BACTERIA

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Abstract –This study was to examine antimicrobial activity of lactic acid solution and crude bacteriocin (pH 4) produced by *Lactobacillus plantarum* KL-1 against *Salmonella* Anatum, *Escherichia coli* and *Staphylococcus aureus.* The experiment conducted into 6 groups including 0, 2, 4% of lactic acid solutions, 2% of crude bacteriocin, the combination of 2% lactic acid solution and 2% crude bacteriocin and combination of 4% lactic acid solution and 2% of crude bacteriocin. Three tested bacteria were inoculated into solution as mentioned above then incubated at 37°C for 24 h. The growth was evaluated at 0, 12 and 24 h. The results showed that 4% of lactic acid solution inhibited the growth of *S.* Anatum, *E. coli* and *S. aureus* after 12 and 24 h. In addition, the combination of 2 and 4% lactic acid solution with 2% crude bacteriocin was highly effective to reduce the growth of all tested bacteria.

Key Words – bacteriocin, lactic acid, pathogenic bacteria

I. INTRODUCTION

Food safety is recognized as one of the most important issues for human health. One of the concerns in food industry is the contamination by pathogens, which are frequently cause by food borne diseases. Foodborne pathogens such as *Salmonella* spp., *E. coli* and *S. aureus* are serious concern in food processing. Over the past decade, recurrent outbreaks of diarrhea, combined with the natural resistance of the causative agents, contributed to its status as hazard [1,2]. Biopreservative systems in foods are of increasing interest for industry and consumers. Lactic acid solution and bacteriocins are considered as safe additives (GRAS) which is useful to control pathogens and spoiling microorganisms in foods [7]. Lactic acid bacteria are able to produce a number of antimicrobial substances such as organic acids, free fatty acids, hydrogen peroxide, diacetyl and bacteriocin, which have the capacity to inhibit the growth of food pathogenic and spoilage organisms [3,5,6]. Bacteriocins are antibacterial proteins or complexed proteins biologically active with antimicrobial action against other bacteria, principally closely related species. Most of the bacteriocins produced by LAB inhibit the growth of other, but some are bactericidal to a number of food pathogens and food-spoilage bacteria [7,10]. Bateriocin produced by *Lactobacillus plantarum* KL-1 showed inhibitory activity against *L. sakei*, *Leuconostoc mesenteroides* and *Enterococcus faecalis* [8]. Therefore the aim of this study was to investigate antibacterial activity of lactic acid solution and crude bacteriocin against meat pathogenic bacteria.

II. MATERIALS AND METHODS

Microorganism

Salmonella Anatum, *E. coli* TISTR 780 and *S. aureus* TISTR 118 were obtained from the Microbiological Resources Center, Thailand Institute of Scientific and Technological Research (TISTR). They were routinely grown in Tryptic soy broth (Merck, Germany) and 0.6% yeast extract (TSB-YE) medium at 37^{o}C for 24 h. *L. plantarum* KL-1, was isolated from traditional Vietnamese fermented pork called "Nem chua" and cultured in MRS broth (de Man Rogasa and Shape; Merck, Germany) at 30 ^{o}C for 14-18 h. The culture was stored at -80 ^{o}C in glycerol final concentration at 15% to use as stock culture.

Crude bacteriocin preparation

Stock cultured was transferred to MRS broth with 1% (w/v) NaCl and incubated over night at 37 ^oC for refresh culture. Then, transferred 2% (v/v) culture in MRS broth again and incubated over night at 37 ^oC. Subsequently, culture broth was centrifuged at 1,500xg at 4 ^oC (Jouan CR3i, France). Cell-free supernatant (CFS) known as crude bacteriocin was sterilized by syringe filter (0.2 µm, Sartorius stedim biotech) without adjust the pH (pH 4).

Effect of lactic acid solution and crude bacteriocin against the growth of S. Anatum, E. coli TISTR 780 and S. aureus TISTR 118

Different concentration of lactic acid solution and crude bacteriocin were added in TSB-YE media for inhibition of microbial growth. The experiment divided into 6 groups; 0, 2 and 4% lactic acid solutions, 2% crude bacteriocin, and the combination of 2 and 4% lactic acid solution with 2% of crude bacteriocin. Culture of 2% pathogenic bacteria was transferred to TSB-YE with different concentration of lactic acid solution and crude bacteriocin as mentioned above. Then, the culture was incubated at 37^oC for 0, 12 and 24 h. The bacteria growth in the culture was determined by spread plate technique using Tryptic soy agar (TSA) (Merck, Germany) and it was expressed in log colony forming unit (log cfu/g)

Statistical analysis

All the experiments were carried out using Completely Randomized Design (CRD). Significant differences between means were separated using Duncan's New Multiple Rang test (DMRT) with SPSS (SPSS 16.0 for windows, SPSS Inc., Chicago, IL, USA).

III. RESULTS AND DISCUSSION

Effect of lactic acid solution and crude bacteriocin on S. Anatum growth

The result displayed that initial growth of all groups had close figure. However, increasing of lactic acid solution concentration was more inhibit the growth of *S.* Anatum. The treatment without lactic acid demonstrated significant growth at 6.26, 7.96 and 8.36 log cfu/ml for 0, 12 and 24 h respectively. Treatment with 4% lactic acid inhibited *S.* Anatum after 12 and 24 h exposure time. The growth was 6.27, 6.25 and 5.97 log cfu/ml for 0, 12 and 24 h respectively. Whereas, using crude bacteriocin at 2% had effect by decreasing the growth number of *S.* Anatum from 6.26 to 5.33 log cfu/ml after 24 h. However, the combination of 2 and 4% lactic acid with 2% crude bacteriocin reduced the growth number of *S.* Anatum after 12 h incubation at 6.23, 5.13 and 4.04 log cfu/ml and 6.21, 4.90 and 3.76 log cfu/ml for 0, 12 and 24 h respectively. The result is shown in table 1.

Table 1 Effect of lactic acid solution and crude bacteriocin on *S.* Anatum growth

Treatment	Number of *S.* Anatum (log cfu/ml)		
	0 h	12 h	24 h
0 % LA	6.26 ^{a,x}	7.96 ^{c,y}	8.36 ^{c,y}
2 % LA	6.30 ^{a,x}	7.24 ^{b,x}	8.01 ^{c,y}
4 % LA	6.27 ^{a,x}	6.15 ^{b,x}	5.97 ^{b,x}
2 % CB	6.26 ^{a,x}	5.95 ^{b,x}	5.33 ^{b,x}
2 % LA + 2 % CB	6.23 ^{a,y}	5.13 ^{b,y}	4.04 ^{a,x}
4 % LA + 2 % CB	6.21 ^{a,y}	4.90 ^{a,x}	3.76 ^{a,x}

Note: LA means Lactic acid solution
CB menas Crude bacteriocin
$^{a-c}$ values with different letters within the same row differ significantly ($p<0.05$)
$^{x-z}$ values with different letters within the same column differ significantly ($p<0.05$)

Effect of lactic acid solution and crude bacteriocin on E. coli TISTR 780 growth

The result in table 2 showed that no effect on *E. coli* TISTR 780 growth when treated with 2% lactic acid compared to control (0% lactic acid), while the reduction of growth appeared at 4% lactic acid and 2% crude bacteriocin after 24 h incubation. The growth of *E. coli* TISTR 780 when treated with 4% lactic acid was at 6.21, 6.18 and 4.81 log cfu/ml and treated with 2% crude bacteriocin was at 6.20, 6.28 and 5.11 log cfu/ml for 0, 12 and 24 h respectively. Whereas, the combination of 4% lactic acid with 2% crude bacteriocin significantly decreased the growth number of *E. coli* TISTR 780 after 12 and 24 h incubation compared to 0 h at 6.19, 5.03 and 3.69 log cfu/ml for 0, 12 and 24 h respectively.

Table 2 Effect of lactic acid solution and crude bacteriocin on *E. coli* TISTR 780 growth

Treatment	Number of *E. coli* TISTR 780 (log cfu/ml)		
	0 h	12 h	24 h
0 % LA	6.17 ^{a,x}	8.23 ^{c,y}	8.38 ^{b,y}
2 % LA	6.12 ^{a,x}	7.15 ^{b,y}	8.12 ^{b,z}
4 % LA	6.21 ^{a,y}	6.16 ^{b,y}	4.81 ^{a,x}
2 % CB	6.20 ^{a,y}	6.28 ^{b,y}	5.11 ^{a,x}
2 % LA + 2 % CB	6.24 ^{a,y}	5.15 ^{a,x}	4.05 ^{a,x}
4 % LA + 2 % CB	6.19 ^{a,z}	5.03 ^{a,y}	3.69 ^{a,x}

Note: LA means Lactic acid solution
CB menas Crude bacteriocin
$^{a-c}$ values with different letters within the same row differ significantly ($p<0.05$)
$^{x-z}$ values with different letters within the same column differ significantly ($p<0.05$)

Effect of lactic acid solution and crude bacteriocin on S. aureus TISTR 118 growth

There was no effect on *S. aureus* TISTR 118 growth when treated with 2% lactic acid compared to control (0% lactic acid). While 4% lactic acid and 2% crude bacteriocin showed decline in growth at 24 h incubation at 6.22, 6.13 and 4.79 log cfu/ml and 6.23, 6.93 and 5.09 log cfu/ml for 0, 12 and 24 h, respectively (Table 3). However, using the combination of 2 and 4% lactic acid with 2% crude bacteriocin, number of *S. aureus* TISTR 118 growth significantly decreased after 12 and 24 h incubation. The growth was at 5.17 and 4.13 log cfu/ml and 4.07 and 3.63 log cfu/ml, respectively.

Table 3 Effect of lactic acid solution and crude bacteriocin on *S. aureus* TISTR 118 growth

Treatment	Number of *S. aureus* TISTR 118 (log cfu/ml)		
	0 h	12 h	24 h
0 % LA	6.28 ^{a,x}	8.22 ^{c,y}	8.36 ^{b,y}
2 % LA	6.27 ^{a,x}	7.21 ^{b,y}	8.17 ^{b,z}
4 % LA	6.22 ^{a,y}	6.13 ^{b,y}	4.79 ^{a,x}
2 % CB	6.23 ^{a,y}	6.93 ^{b,y}	5.09 ^{a,x}
2 % LA + 2 % CB	6.27 ^{a,z}	5.17 ^{a,y}	4.13 ^{a,x}
4 % LA + 2 % CB	6.21 ^{a,z}	4.07 ^{a,y}	3.63 ^{a,x}

Note: LA means Lactic acid solution
CB menas Crude bacteriocin
$^{a-c}$ values with different letters within the same row differ significantly ($p<0.05$)
$^{x-z}$ values with different letters within the same column differ significantly ($p<0.05$)

The concentration of 4% lactic acid solution and 2% crude bacteriocin inhibited the growth of *S*. Anatum, *E. coli* TISTR 780 and *S. aureus* TISTR 118 at 12 and 24 h incubation. Lactic acid can disturb pH homeostasis of bacteria resulting in stressed cells. In the undissociated form, lactic acid and other weak cell acids are lipolytic, which enable acid molecules to freely diffuse across the bacterial cell membrane. One side, the acid may dissociate and release protons that acidify the cytoplasm. Energy is diverted to maintain internal pH, and hence, growth is reduced or inhibited diarrhea [8]. Lactic acid (0.5%) could completely inhibit the growth of *S.* Enteritidis, *E. coli* and *L. monocytogenes* due to the leakage of proteins in those bacteria [10]. The inhibition mechanism of lactic acid is related to the reduction in pH, cell physiology and metabolisms. It is known that reduction levels of pathogens following treatment with lactic acid increased with highly lactic acid concentration [1]. Researcher studied on inhibition of foodborne bacterial pathogens by bacteriocin form lactic acid isolated from meat and they found that bacteriocin from eight isolates out of ten had inhibitory activity against pathogenic bacteria including *Listeria monocytogenes*, *Aeromonas hydrophila* and *S. aureus* [11]. In this study, the combination of lactic acid solution and crude bacteriocin showed higher inhibitory activity than individual treatment.

IV. CONCLUSION

Using 4% lactic acid solution or 2% crude bacteriocin produced by *L. plantarum* KL-1 could clearly reduce the growth of all tested bacteria after 24 h. incubation. However, the combination of 2 and 4 % lactic acid with 2% crude bacteriocin decreased the growth of all tested bacteria earlier at 12 h incubation. Therefore, to kill and remove pathogenic microorganisms from food, it would be advantage to apply the combination of lactic acid solution and crude bacteriocin which might provide the greater inhibitory effect.

ACKNOWLEDGEMENTS

The authors would like to thank Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang for support the research financial.

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