Screening and Selection of Lactic Acid Bacteria with High Antimicrobial Activity for Fermented Sausage Production in Vietnam

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Abstract— **Nowadays, fermented meat products in Vietnam are still poor in variety, quantity, quality and especially safety. They are usually produced manually with natural fermentation process and high risk raw materials particularly regarding pathogenic contamination.**

In this work, the fermentation ability, the resistance to NaCl and $NaNO_2$, and particularly the antimicrobial activity against *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* K12TG1 of six lactic acid bacterial strains (namely bacteriocin producing *Lactobacillus plantarum* H1.40, sakasin A producing *L. sakei* DSM 6333, *L. curvatus* DSM 20019, *S. xylosus* 2032, and two commercial meat starter *Miccrococcus* spp. CP1 and *Lactobacillus* spp. CP2) were examined. Two strains (H1.40 and DSM 6333) having the best antimicrobial and technological properties were then selected and used as mix starter culture (ratio 1:1) for fermented sausage production.

Results showed that after 96 h of fermentation, the pH values of fermented sausages using selected starter strains decreased to 4.7 - 4.8, whereas the pH of those using commercial meat starter decreased to 4.9, and the pH of the control (without starters) remained around 5.2. Furthermore, the sausages using starter culture mix were 1.7 log CFU/g of *S. aureus* and 2.1 log CFU/g of *Enterobacteriaceae* lower than the control, which is better than those using commercial starter culture, i.e. only 1 log CFU/g of *S. aureus* and 1.4 log CFU/g of *Enterobacteriacea* lower than the control.

Our study demonstrated that the use of these selected strains as starter culture in fermented sausage manufacturing could improve product quality and microbiological safety and stability.

Keywords— **fermented sausage, lactic acid bacteria, starter culture.**

I. INTRODUCTION

Nowadays, fermented meat products in Vietnam are quite poor in types as well as in quantity; most of them are produced manually using the natural fermentation process, leading to unstable product quality. Especially the raw meat materials have a high risk of contamination of pathogenic microorganisms belonging to the family of *Enterobacteriaceae*, *Staphylococcus aureus*, *Salmonella* spp., etc. during butchering, transporting and manual storage. Therefore, the selection of bacterial strains which have good biological activity as well as high antibacterial (bacteriocin-producing) and good fermentation ability, etc. is very necessary to obtain a good starter culture for production of fermented meat products in order to industrialize the production, control product quality and ensure food safety [1], [3], [11], [12].

II. MATERIALS AND METHODS

The experimental strains: Lactobacillus plantarum H1.40 isolated from Vietnamese fermented pork (nem chua) producing bacteriocin [8]; *L. sakei* DSM 6333 producing sakasin A (collection strain); *L. curvatus* DSM 20019 (collection strain); *S. xylosus* 2032 isolated from natural fermented sausages (from Torino University); *Micrococcus* spp. CP1 and *Lactobacillus* spp. CP2 isolated from commercial meat starter (Cargill France SAS, Starter cultures GY2/100kg).

Indicator organism: S. aureus ATCC 25923, *Escherichia coli* K12TG1 (from ENSBANA University).

Fermented sausages were prepared using the following recipe for Pepperoni [10] (for 100 kg): 50 kg pork trimmings; 30kg beef chucks, cheeks; 20kg pork jowl; NaCl: 2.8kg; $NaNO_2$ 0.015kg, Glucoza: 0.8kg; Chili: 0.05kg; Ground pepper: 0.6kg; Peeled garlic: 0.03kg; Monosodium glutamate: 0.12kg.

Processing and handling: Pork and beef were ground using first a 10–12 mm plate and then a 3 mm plate. The seasoning was prepared in a mixer for at least 5 minutes before adding to the ground meat. The mixture was stuffed directly into 28 to 32-mm

diameter casings (pig intestines). The sausages were held at 22–24°C, relative humidity of 85% for 48 hours, and then at 32–34°C for 2 days. The sausages were further dried at 14°C for 15 days.

The antibacterial activity of the bacterial strains was determined using agar diffusion method [2].

Enterobacteriaceae was enumerated using F18 standard method of UK [6].

S. aureus was enumerated using TCVN 4830-1: 2005 (ISO 6888-1:1999, Amd 1: 2003) method.

III. RESULT AND DISCUSSION

A. Physiological and biochemical characteristics of lactic acid bacteria (LAB) strains for fermented sausage.

1. Growth of LAB at different temperatures

Six LAB strains were cultured in static MRS broth at 25, 30 and 37°C. Their growth ability was evaluated by measuring optical density (OD) at 620 nm. The results are shown in Table 1

Table 1. The growth of experimental strains at different temperature

Activity of strains	25°C	30°C	37°C
L. sakei DSM 6333	+++	+++	++
L. curvatus DSM 20019	+++	++	++
S. xylosus 2032	++	++	+
L. plantarum H1.40	++	+++	+++
Micrococcus spp. CP1	++	+	+
Lactobacillus spp. CP2	++	+	+

+++: very strong growth; ++: good growth; +: weak growth

Temperature of 25°C is the most suitable for the growth of *L. sakei* DSM 6333 and *L. curvatus* DSM 20019. At 30°C, 37°C, the strain H1.40 exhibited the strongest growth, followed by *L. sakei* DSM 6333. The two strains in commercial preparation (CP1 and CP2) did not grow well at the temperature ranging from 30°C to 37°C, the most common temperature range in Vietnam.

2. Antibacterial activity of LAB strains

Table 2. The antibacterial ability (D-d) mm of experimental strains with *S. aureus* and *E. coli*

Strains	with *S. aureus* (MRS)	with *S. aureus* (MRS -Mo)	with *E. coli* (MRS)	with *E. coli* (MRS -Mo)
L. sakei DSM 6333	16	13	12	10
L. curvatus DSM 20019	15	11	11	8
S. xylosus 2032	11	8	6	4
L plantarum H1.40	17	15	10	8
Micrococcus spp. CP1	9	5	5	2
Lactobacillus spp. CP2	6	3	0	0

MRS (Mo): MRS added NaCl 2%, $NaNO_2$ 200ppm

The antibacterial ability of six test LAB strains is shown in Table 2. It appears that all test strains exhibited antibacterial ability with different levels towards all of the indicator strains, with the descending order as following: DSM 6333 ≈ H1.40 > DSM 20019 > 2032 > CP1 > CP2 (Table 2). The antibacterial ability reduced 10 to 20% in medium supplemented with 2% NaCl and 200 ppm $NaNO_2$, with the strain H1.40 least affected by the addition.

The strains *L. plantarum* H1.40, *L. sakei* DSM 6333 and *L. curvatus* DSM20011 were thus selected for their strong antibacterial ability, especially towards *S. aureus* and *E. coli*, the two common contaminating microorganisms in meat products. They are also suitable for use as starter culture in producing fermented sausage in Vietnam due to their good lactic fermentation in the temperature range from 25 - 37°C and resistance to NaCl and $NaNO_2$.

B. Production of Pepperoni using the selected LAB

Four samples of Pepperoni were produced as described above using a mixture of two LAB strains (ratio of 1:1) with density of 10^6 CFU/g of sausage as follows: Sample I: *L. sakei* DSM 6333 and *L. curvatus* DSM20019; Sample II: *L. plantarum* H1.40 and *L. curvatus* DSM20019; Sample III: *L. sakei* DSM 6333 and *L. plantarum* H1.40; Sample IV: *Micrococcus* spp.CP1 and *Lactobacillus* spp.CP2 (commercial starter culture); Sample V: Control (no starter culture).

The quality of fermented sausages was evaluated based on product pH, LAB, *Enterobacteriaceae* and *S. aureus* counts. These results are showed in Figure 1-4.

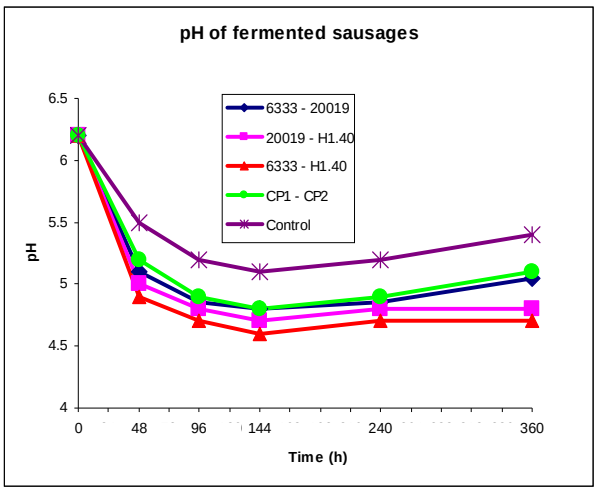

Fig. 1 pH of fermented sausages

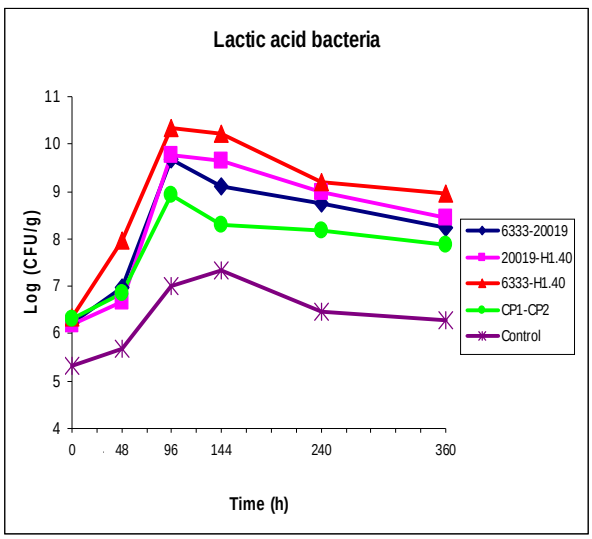

Fig. 2 Number of lactic acid bacteria of fermented sausages

pH of the sausages with added starter culture decreased quickly, reaching 4.7 to 4.8 after 48 and 96 hours, showing the good direction of the lactic fermentation process. The sausage samples using the commercial starter culture (sample IV) and control (sample V) had higher pH values (4.9 and 5.2 after 48 hours, respectively). The decrease in pH is almost correlated with the amount of LAB in the sausages, i.e. 9-10 logs CFU of LAB per g of sausages using starter culture (sample I to IV) compared to max 7 log CFU of LAB per g of control sausage (sample V). The lower pH is favourable in producing fermented sausages for Vietnamese market compared to European ones due to the risk of microorganism contamination in raw meat.

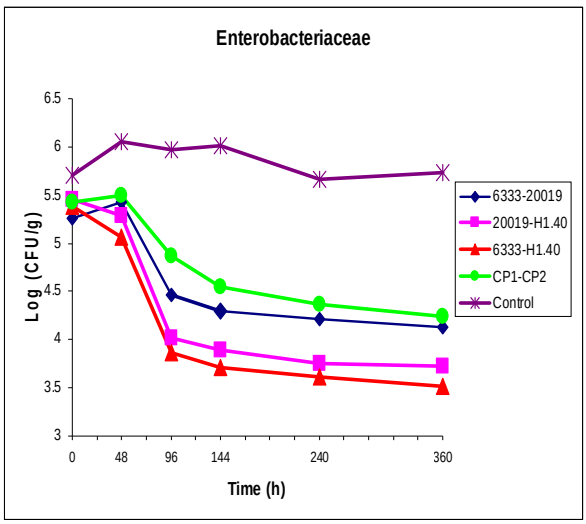

Fig. 3 Number of *Enterobacteriaceae* of fermented sausages

Figures 3 and 4 show that the loads of *Enterobacteriaceae* and *S. aureus* in fresh sausages samples were quite high but reduced evidently after 96 hours of fermentation. The reduction was most remarkable in sausage fermented by DSM 6333 and H1.40 (sample III). This sausage sample had 1.7 log CFU/g of *S. aureus* and 2.1 log CFU/g of *Enterobacteriaceae* lower than the control, which is better than those using commercial starter culture, i.e. only 1.0 log CFU/g of *S. aureus* and 1.4 log CFU/g of *Enterobacteriacea* lower than the control. This result is in accordance with data reported by Kaban. G [7], Pidcock [9] and Visessanguan [13]. Sample III also met the requirements by Australia New Zealand Food Standards for *Enterobacteriaceae* ($<10^4$ CFU/g) and *S. aureus* ($<10^3$ CFU/g) [4], [5].

The sausages with starter culture also showed better sensory characteristics with firm texture and balanced taste compared to the control samples to the orientation and control of the fermentation process.

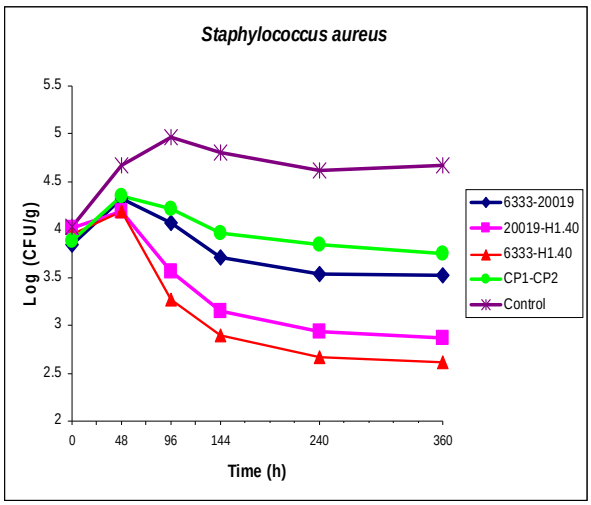

Fig. 4 Number of *S. aureus* of fermented sausages

IV. CONCLUSIONS

Two strains of LAB having strongest antibacterial activity namely *L. sakei* DSM 6333 and *L. plantarum* H1.40 were selected and used as starter culture for fermented sausages using Pepperoni formula. The quality of these fermented sausage is significantly improved on all indicators, particularly regarding microbial loads, i.e. 1.7 - 2 log CFU/g of *S. aureus* and 2.1 - 2.2 log CFU/g *Enterobacteriaceae* lower than the control samples (at 96h and 360h). The use of starter culture also enhanced the product safety to meet Australia New Zealand Food Standards. The finding of this study is thus highly appreciated as it provides a solution to improve safety and quality of fermented sausages in Vietnam and introduce new sausage variety into local market.

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