BIOGENIC AMINE INHIBITION OF HARBIN DRY SAUSAGE BY INOCULATION WITH *STAPHYLOCOCCUS XYLOSUS* AND *LACTOBACILLUS PLANTARUM*

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Abstract

This study was conducted to evaluate the effects of inoculation with *Staphylococcus xylosus*, *Lactobacillus plantarum*, or a mixture of strains (*L. plantarum* + *S. xylosus*) on the formation of biogenic amines (BAs) and bacterial counts in Harbin dry sausage. The results showed that *L. plantarum* and *S. xylosus* could inhibit BA accumulation and restrain enterobacteriaceae growth. These results demonstrate that *S. xylosus* and *L. plantarum* could be used as starter cultures in Harbin dry sausage production to inhibit BA accumulation.

Keywords: Biogenic amines, Harbin dry sausage, Bacterial strains

I. INTRODUCTION

Biogenic amines (BAs) are organic, basic, nitrogenous compounds of low molecular weight, formed by decarboxylation of amino acids via microbial actions. An excess of BAs in the body is hazardous to the nervous and cardiovascular systems, leading to physical discomforts [1]. In addition, BAs are the precursors of nitrosamines, which are carcinogenic.

Lactobacillus and *Staphylococcus* strains have been widely studied and used as starter cultures in meat products. However, there is also a risk in using these bacterial starter cultures due to excessive accumulation of BAs during sausage fermentation.

The objective of this study was to evaluate the effect of starter cultures of amine-negative *S. xylosus* and *L. plantarum* (proved in our previous studies) on the accumulation of BAs in Harbin dry sausages, meanwhile the bacterial counts was also evaluated.

II. MATERIALS AND METHODS

1. Bacterial cultures

S. xylosus was isolated from Harbin dry sausage and identified by sequencing of 16S rDNA [2]. *L. plantarum* was obtained from Key Laboratory. *L. plantarum* was kept on Man Rogosa and Sharp (MRS) agar plates and *S. xylosus* was kept on Mannitol Salt Agar (MSA) plates at 4 °C. After 12 h of cultivation in MRS and MSA broth, the *L. plantarum* and *S. xylosus* cells were collected by centrifugation (9,000 × *g*) with a centrifuge for 10 min at 4 °C, washed twice with saline solution, and resuspended in the same solution at a concentration of 10^7 CFU/mL. These suspensions were used as starter cultures.

2. Preparation of Harbin dry sausage

Four batches of Harbin dry sausages were manufactured (control; *L. plantarum*; *S. xylosus*; and *L. plantarum* + *S. xylosus*). The sausages were prepared as described by Chen *et al.* [3] with slight modifications, changes as the follows: after mixing thoroughly, the meat batter was inoculated with *L. plantarum* or *S. xylosus* suspensions. Samples were collected on days 0, 3, 6, and 9; four sausages per batch were used for analysis.

3. Bacterial counts

Microbiological analyses were performed immediately after the stuffing and during the fermentation period as described by Wang *et al.* [4] with slight modifications. Total aerobic counts (TACs) were measured using plate count agar after incubation at 37 °C for 48 h. LAB counts were enumerated on MRS agar after incubation at 30 °C for 48 h. Enterobacteriaceae

counts were cultured on crystal-violet neutral-red bile dextrose agar plates at 37 °C for 48 h.

4. Biogenic amines

The extraction of BAs from samples was performed according to the method of Hong *et al*. [5]. The analysis of BAs was performed according to the method of Latorre-Moratalla *et al*. [6].

5. Statistical analysis

All the data were analyzed statistically using the General Linear Models procedure of the Statistix 8.1 software package (Analytical Software, St Paul, MN, USA), and presented as mean ± standard deviations (SD). One-way analysis of variance (ANOVA) with the Tukey's multiple comparison was used to measure the significance of the main effects ($P < 0.05$).

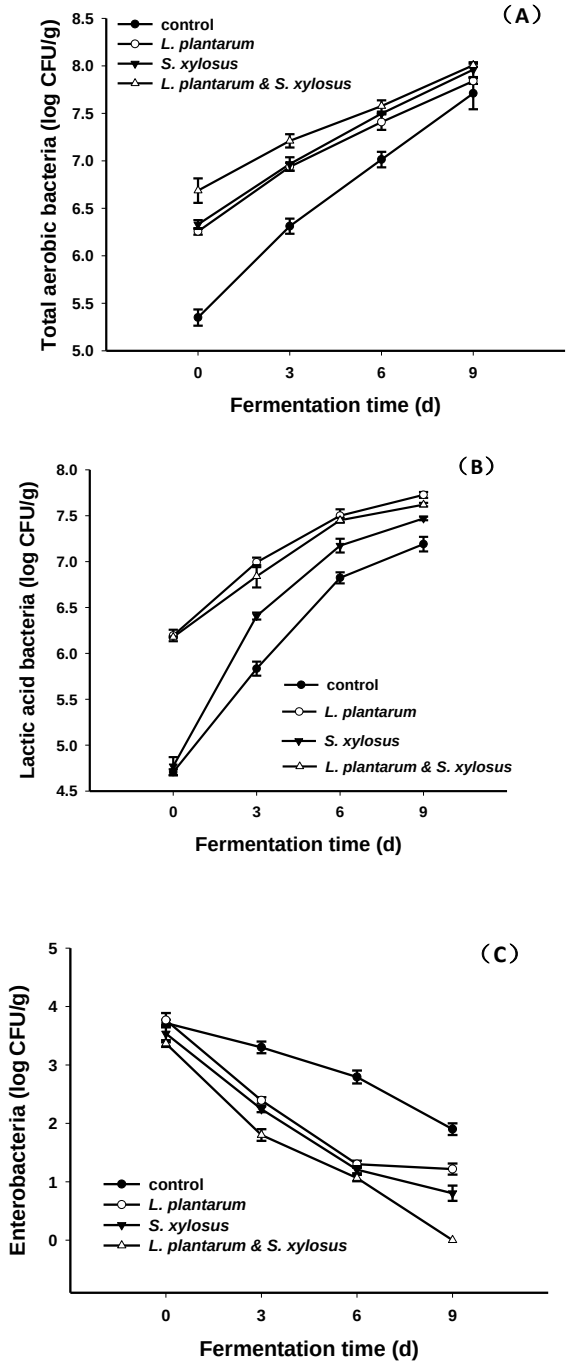
III. RESULTS AND DISCUSSION

1. Bacterial counts

The bacterial counts in dry sausages that were not inoculated and in those that were inoculated with different bacterial strains are shown in Fig. 1. The TACs and LAB counts increased during fermentation; LAB counts in all of the sausages increased sharply and became the predominant microorganism throughout fermentation, reaching maximum levels at day 9 ($P < 0.05$). LAB counts in sausages inoculated with a starter culture that included *L. plantarum* increased faster than those in the control sausages and in sausages inoculated only with *S. xylosus* ($P < 0.05$). The TACs were higher in the inoculated sausages than in the control during the first 6 days, and this may be attributed to changes in the LAB counts. However, the differences in the final levels of TACs among all of the sausages were not significant ($P > 0.05$).

In contrast, enterobacteriaceae counts decreased significantly during fermentation , independent of the presence of starter cultures ($P < 0.05$). Enterobacteriaceae counts in the inoculated sausages decreased faster than those in the control, especially in mixed strains bath ($P < 0.05$). The results showed that the starter culture can effectively inhibit the growth of enterobacteriaceae.

Figure 1. Changes in TACs (A), LAB (B), and enterobacteriaceae (C) counts (log CFU/g) in dry sausages inoculated with various strains during fermentation.

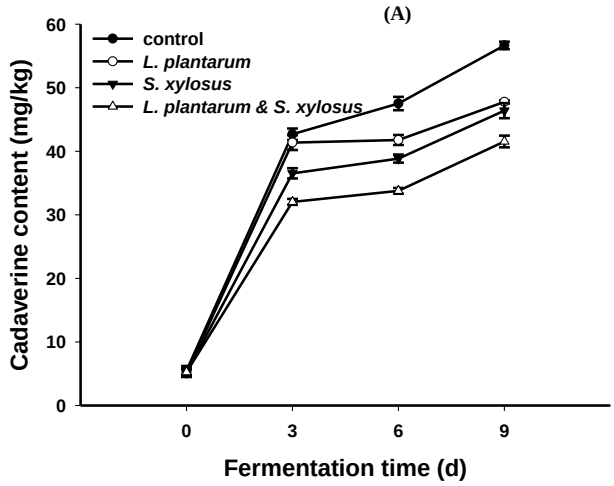


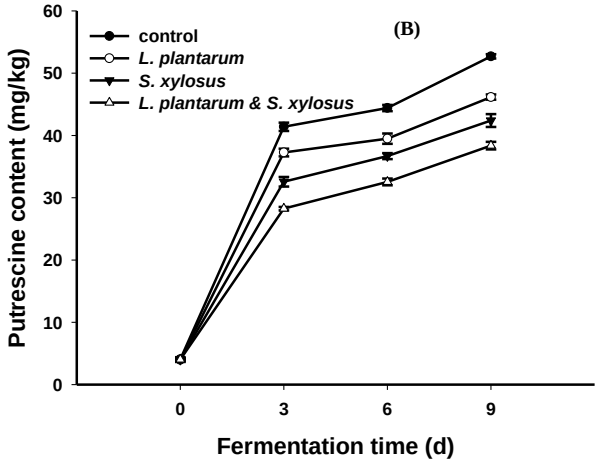
2.Biogenic amines

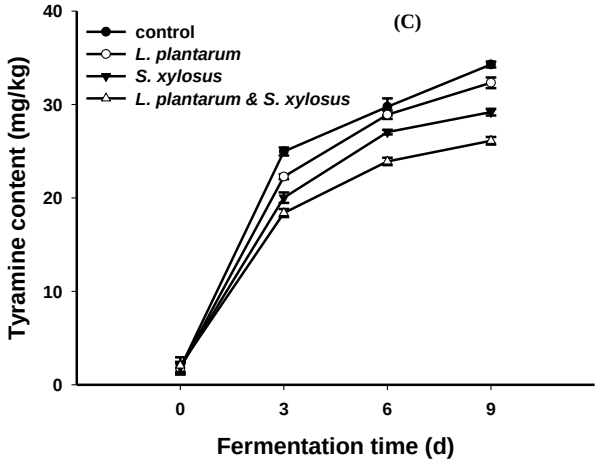
Cadaverine concentrations significantly increased during fermentation ($P < 0.05$, Fig. 2A). The lowest concentrations were observed in sausages inoculated with the bacterial mixture ($P < 0.05$), which can be explained by the low enterobacteria counts. Putrescine is another primary BA found in dry sausages, and changes in its concentration were similar to those of cadaverine. As shown in Fig. 2B, the putrescine concentrations increased rapidly during the initial 3 days, increasing to 50.53, 44.11, 40.85, and 36.03 mg/kg at day 9 for the control sausages and sausages inoculated with *L. plantaru*, *S. xylosus*, and a mixture of these strains, respectively ($P < 0.05$).

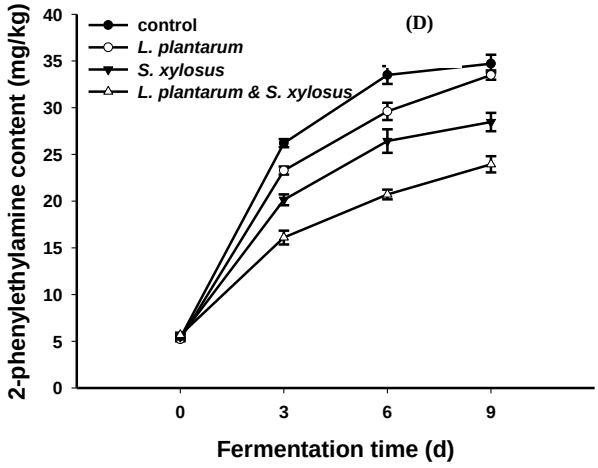
As shown in Fig. 2C, the tyramine concentrations in the sausages inoculated with *L. plantarum*, *S. xylosus*, and a mixture of these strains were 4.8%, 14.3%, and 23.4% lower, respectively, than that in the control sausage at day 9 ($P < 0.05$). The 2-phenylethylamine increased rapidly during fermentation (Fig. 2D). Compared to the control, there was a 31.15% reduction in the 2-phenylethylamine concentration at day 9 ($P < 0.05$) in sausage inoculated with a mixture of bacterial strains.

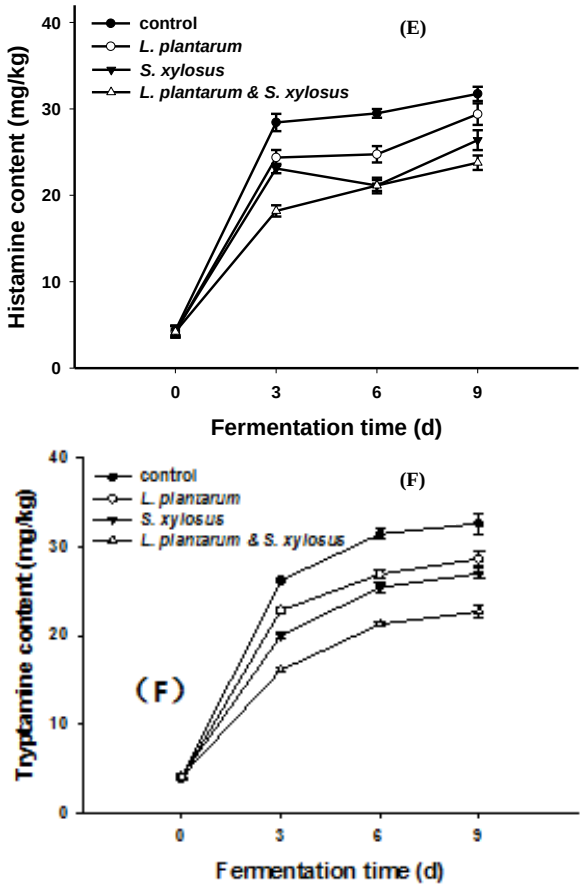
Figure 2. Changes in the amounts of cadaverine (A), putrescine (B), tryptamine (C), 2-phenylethylamine (D), histamine (E), and tyramine (F) (mg/kg) in dry sausages inoculated with various strains during fermentation.











As shown in Fig. 2E, the histamine concentrations in the inoculated sausages were much lower than that in the control. At day 9 of fermentation, the histamine concentration in sausages inoculated with the mixed starter culture was 25.6% lower than that of the control ($P < 0.05$). The tryptamine concentration in sausages inoculated with the mixed starter culture was 30.4% lower than that of the control at day 9 ($P < 0.05$, Fig. 2F).

IV. CONCLUSION

The TAC and LAB counts were higher in the inoculated sausages, especially in those inoculated with a mixture of bacterial strains (*S. xylosus* + *L. plantarum*). However, the growth of enterobacteriaceae was inhibited in these sausages. Inoculation with bacterial strains, especially a mixture of strains, could decrease the accumulation of BAs.These results suggest that *S. xylosus* and *L. plantarum* could be considered as candidates for a mixed bacterialstarter culture in dry sausage production to inhibit BA accumulation.

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