Degrading sarcoplasmic protein and biogenic amine by lactic acid bacteria isolated from Sichuan traditional sausage

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I. INTRODUCTION

The breaking down of protein into polypeptides and free amino acids during sausage fermentation is generally related to products' characteristic taste and flavor. Lactic acid bacteria (LAB) are the dominant microorganism growing during sausage fermentation, and certain strains have shown a capacity for proteolysis in the meat protein extract model system [1]. However, the free amino acids can form biogenic amines (BAs) by the action of decarboxylases [2]. Fermented sausages usually contain relatively high level BAs, among which the major ones are tryptamine (TRY), ß-phenylethylamine (PHE), putrescine (PUT), cadaverine (CAD), histamine (HIS), tyramine (TYR), spermidine (SPD) and spermine (SPM).It is well known that high level of BAs can elicit toxic effects in humans. Some LAB have shown a capacity of degrading BAs. Therefore, it is of great significance to screen LAB strains that can degrade both sarcoplasmic protein and BAs to give favored flavor but maintain relatively low level of BAs.

Sichuan traditional sausage is produced by spontaneous fermentation and has been accepted widely in China. Our previous study has screened LAB strains of high potential, including L1: *L. plantarum* 1.1, L2: *Weissella hellenica*, L3: *Leuconostoc mesenteroides*, L4: *L. curvatus*, L5: *L. plantarum* and L6: *W. viridescens*, isolated from Sichuan traditional sausage. The object of this study was to determine the degradation activity on sarcoplasmic protein as well as BAs by these LAB strains screened..

II. MATERIALS AND METHODS

Protein analysis by electrophoresis. LAB cells were added to sarcoplasmic protein extracts (10^7 cfu/mL), with the extract without bacterial cells used as a control. The mixture was inoculated at 37 ℃for 4 days, and sampled every day for sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). A 12% acrylamide resolving gel and a 5% acrylamide stacking gel were used.

Evolution of biodegradability of BAs. LAB cells were re-suspended in sodium phosphate buffer which contained 100 mg/L each of the eight Bas (10^7 cfu/mL). The cell suspensions were incubated at 37 ℃ for 24 h. The buffer without any bacterial cells was used as a control. After incubation, the cell suspensions centrifuged and the supernatant was derivatized with dansyl chloride, and the high performance liquid chromatography (HPLC) determination of dansyl derivatives of BAs were performed with a Waters liquid chromatography system.

III. RESULTS AND DISCUSSION

Protein degradation. The electrophoretic patterns derived from hydrolysis of sarcoplasmic proteins by different LAB are shown in Figure 1. There was almost no variation in control during the first 3 days. Control samples, lacking any bacterial enzyme, reflected the activity of endogenous proteinases responsible for the decrease in intensity of protein bands at approximately 63, 40 and 25 kDa on day 4. The band changes of the samples inoculated with L3 were similar to the control with only slight degradation of the bands. The bands of the samples inoculated with L1 were changed obviously on day 3 and day 4, and the bands at approximately 60, 48, 30 and 25 kDa were disappeared. The protein profile in other LAB-treated samples significantly changed at day 4. The above results showed that the degradation of sarcoplasmic protein was mainly caused by microbial enzymes instead of muscle proteinase, which was absence here.

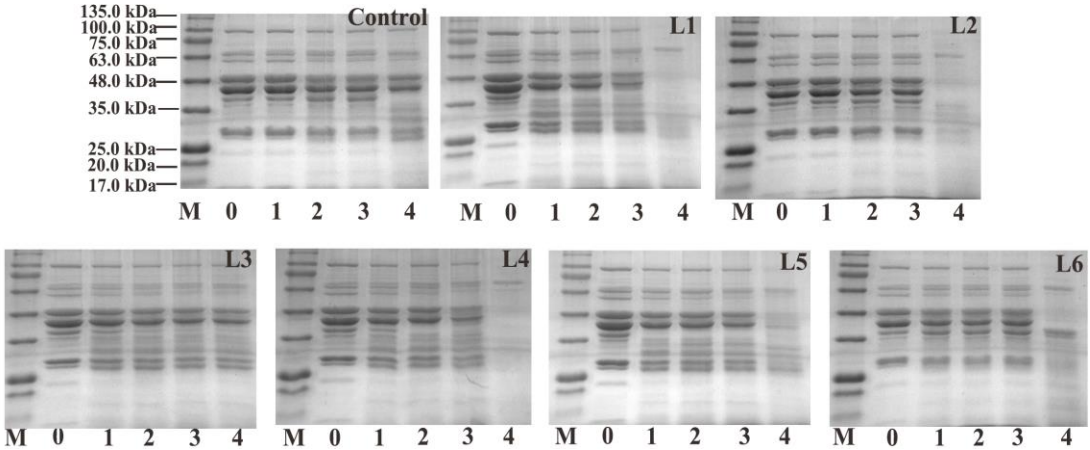

Figure 1. SDS-PAGE pattern changes in sarcoplasmic proteins inoculated with LAB strains during incubation at 37 °C; M refers protein Marker; 0-4 refers to time of incubation (days).

Biodegradation of BAs. Table 1 show the degradation of BAs by LAB. L1 had a certain degradation effect on these 8 kinds of BAs. The degradation rates of these 8 kinds of BAs were all higher than 10%, and L1 had the highest degradation rate of PUT to 16.55%. L4 could degrade the remaining 7 kinds of BAs except TRY. The remaining LAB could only degrade some of the 8 kinds of BAs. L2, L3 and L5 had effect on degrading PUT, CAD, SPD and SPM. L6 had effect on degrading TRY, PHE, PUT and CAD.

Table 1 The biogenic amines degradation rate of different LAB strains

BAs	degradation rate（%）					
	L1	L2	L3	L4	L5	L6
TRY	10.82 ± 109^{Ad}	0.00 ± 0.00^{Be}	0.00 ± 0.00^{Be}	0.00 ± 0.00^{Bi}	1.28 ± 0.74^{Be}	0.00 ± 0.00^{Be}
PHE	11.26 ± 0.24^{Ad}	0.00 ± 0.00^{De}	0.00 ± 0.00^{De}	$4.96 \pm 0.39B^{Dde}$	2.75 ± 0.88^{Cc}	0.00 ± 0.00^{De}
PUT	16.55 ± 0.52^{Ba}	12.27 ± 0.53^{Cb}	11.89 ± 0.51^{Ca}	25.76 ± 1.31^{Aa}	14.96 ± 0.25^{Ba}	23.03 ± 1.87^{Aa}
CAD	13.97 ± 0.74^{Bbc}	4.81 ± 0.90^{Cd}	1.87 ± 0.57^{Dd}	17.24 ± 1.07^{Ac}	5.93 ± 1.38^{Cb}	12.82 ± 0.57^{Bc}
HIS	11.86 ± 1.06^{Acd}	0.00 ± 0.00^{Ce}	0.00 ± 0.00^{Ce}	5.63 ± 0.28^{Bd}	0.00 ± 0.00^{Ce}	0.00 ± 0.00^{Ce}
TYR	14.41 ± 1.47^{Aa}	0.00 ± 0.00^{Ce}	0.00 ± 0.00^{Ce}	4.19 ± 0.53^{Be}	0.00 ± 0.00^{Ce}	0.00 ± 0.00^{Ce}
SPD	13.91 ± 1.52^{Cbc}	10.63 ± 1.40^{Dc}	4.95 ± 0.06^{Ec}	19.36 ± 0.55^{Ab}	0.00 ± 0.00^{Fe}	16.75 ± 1.17^{Bb}
SPM	12.28 ± 0.82^{Bbcd}	14.35 ± 0.42^{Aa}	11.25 ± 0.46^{Cb}	2.62 ± 0.06^{Ef}	0.00 ± 0.00^{Fe}	8.35 ± 0.78^{Dd}

$^{A-F}$ Means within the same row with different uppercase letters differ significantly ($P < 0.05$).
$^{a-i}$ Means within the same column with different lowercase letters differ significantly ($P < 0.05$).

IV. CONCLUSION

L. plantarum 1.1 isolated from traditional Sichuan sausages could simultaneously degrade sarcoplasmic protein and major eight BAs, thus to be potentially used for improving the quality and safety of the fermented meat products.

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