# Role of *Lactobacillus* spp. and *Staphylococcus xylosus* in meat: conversion of metmyoglobin and inhibition of spoilage bacteria

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**Abstract –The conversion of metmyoglobin ($MbFe^{III}$) to red myoglobin derivative by bacteria and their effect on the inhibition of spoilage organisms in raw meat batters during refrigeration were investigated. PCR-denaturing gradient gel electrophoresis was used to reveal the microbial populations. The results showed that *Lactobacillus curvatus*, *Lactobacillus plantarum*, *Staphylococcus xylosus* and *Lactobacillus fermentum* could convert $MbFe^{III}$ into red myoglobin derivatives in model systems, whereas *Lactobacillus sake* and *Pediococcus pentosaceus* could not. *Staphylococcus* spp., *Carnobacterium* sp., *Lactobacillus* sp., *Brochothrix thermosphacta* and *Enterococcus faecalis* were prevalent in control and nitrite-cured meat batters in tray packaging during refrigeration, whereas lactic acid bacteria and Staphylococcus were observed to be the predominant microbial flora in the samples inoculated with *L. sake* and *S. xylosus*, respectively. Most of the spoilage bacteria were inhibited by the two strains. This study provides a potential method for improving meat colour and inhibiting spoilage bacteria via microbial fermentation in meat products.**

## I. INTRODUCTION

Nitrite is a key ingredient in meat curing. It can react with myoglobin (Mb) to form nitrosylmyoglobin ($MbFe^{II}NO$) which is responsible for the characteristic pink colour of cured meat, inhibit unwanted bacteria, retard lipid oxidation and develop desired meat flavours. Unfortunately, nitrite addition can also result in the formation of N-nitrosamines. Therefore, many attempts have been made to find a way to replace nitrite in the meat industry (1). However, only few substitutes have been used widely in meat manufacturing until now (2).

Recently, microbial conversion of metmyoglobin ($MbFe^{III}$) to red myoglobin derivatives was observed. Many bacteria, such as *Lactobacillus fermentum, Lactobacillus plantarum* and *Staphylococcus xylosus*, were proved to have the ability to convert metmyoglobin to red myoglobin derivatives both in model systems and in meat products (3). Besides, many types of bacteria, can inhibit pathogens and/or spoilage microorganisms in food matrices. It has been assumed that if bacteria strains could be found to perform the two functions, or if strains that perform a single function individually were mixed together as a mixed starter culture, the implications would be interesting and beneficial for meat manufacturing.

The objective of this study was to assess the ability of bacteria to convert $MbFe^{III}$ in a model system and to inhibit spoilage bacteria in raw meat batters during refrigeration.

## II. MATERIALS AND METHODS

### *2.1. Bacterial strains and culture media*

*Lactobacillus curvatus*, *L. plantarum*, *Pediococcus pentosaceus* and *S. xylosus* were isolated from Harbin dry sausage. *Lactobacillus sake* and *L. fermentum* were obtained from the China General Microbiological Culture Collection Center.

### *2.2. $MbFe^{III}$ conversion in model system*

A 20-mg/mL $MbFe^{III}$ solution was prepared according to Arihara et al. (4). Bacteria cultures in the log phase were inoculated into 2 mL of MRS broth containing $MbFe^{III}$ to reach an initial concentration of $10^7$ CFU/mL. After 18 h of anaerobic cultivation at 37 °C, the media culture was centrifuged to remove the cells and then the colour and UV-Vis spectra were determined.

### *2.3. Colour measurement*

Colour difference was determined using a ZE-6000 colourimeter (Nippon Denshoku, Kogyo Co., Tokyo, Japan).

*2.4. UV–Vis absorption spectroscopy*

UV–Vis absorption spectra were measured from 450 to 700 nm in 1-nm intervals using a UV–Vis spectrophotometer.

*2.5. Preparation of raw meat batters*

Eight groups of different meat batters were prepared, including the control (C), the nitrite treatment (N) and six treatments each inoculated with one kind of the bacteria strains (Table 1). LAB strains and *S. xylosus* were inoculated at levels of $10^7$ and $10^6$ CFU/g meat, respectively. All of the meat samples were placed in polypropylene trays, wrapped with an oxygen-permeable polyvinyl chloride film and stored at 4 °C for 12 d.

Table1 Formulations of meat batters.

| Samples | Meat 100 g | Bacteria | Nacl 3.0 g | Nitrite 10 mg | Glucose 0.2 g |
|---|---|---|---|---|---|
| C | + | - | + | - | - |
| N | + | - | + | + | - |
| SX | + | *S. xylosus* | + | - | + |
| LS | + | *L. sake* | + | - | + |
| LC | + | *L. curvatus* | + | - | + |
| LP | + | *L. plantarum* | + | - | + |
| PP | + | *P. pentosaceus* | + | - | + |
| LF | + | *L. fermentum* | + | - | + |

*2.6. DNA extraction*

DNA was extracted using the TIANamp Bacteria DNA Kit according to the manufacturer's protocol for the purification of genomic DNA.

*2.7. PCR-DGGE*

Primers U968f-GC containing a GC clamp and L1401r were used to amplify the V6-V8 regions of the bacterial 16S rRNA gene (5). PCR amplicons were separated by DGGE, using the Dcode™ Universal Mutation Detection system. The bands were excised and then amplified, purified and sent to the Beijing Gene Institute for sequencing.

*2.8. Statistical analysis*

Analysis of variance was performed to determine significance. Significant differences ($P < 0.05$) among means were identified using Tukey procedures.

## III. RESULTS AND DISCUSSION

*3.1. $MbFe^{III}$ conversion in model system*

The colour analysis results showed that the samples inoculated with *L. curvatus*, *L. plantarum*, *S. xylosus* and *L. fermentum* had significantly higher $a^*$ values than those of the other samples (Fig. 1; $P < 0.05$), which meant that the samples inoculated with these four strains became much redder in colour. The difference was also noticeable visually. The colour of the MRS model systems inoculated with *L. curvatus*, *L. plantarum*, *S. xylosus* and *L. fermentum* turned from brown to red within 18 h of incubation, whereas the control sample or the samples with added *P. pentosaceus* and *L. sake* still maintained its brown colour.

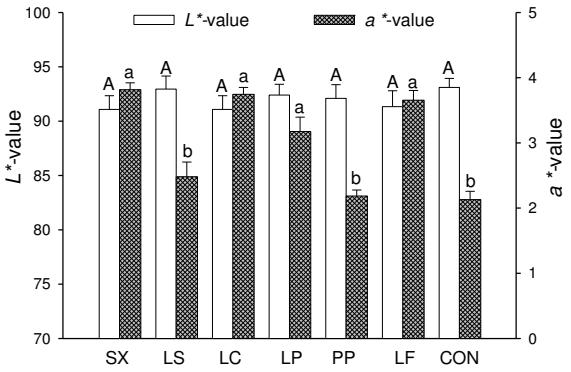


Fig. 1. Transmittance colourimetry of $MbFe^{III}$-MRS broth inoculated with *S. xylosus* (SX), *L. sake* (LS), *L. curvatus* (LC), *L. plantarum* (LP), *P. pentosaceus* (PP) and *L. fermentum* (LF). Means of $a^*$ values with different lowercase letters (a to b) differ significantly ($P < 0.05$). Means of $L^*$ values with the same letter (A) are not significantly different at the 5% probability level.

It was evident from UV–Vis spectral analysis that samples treated with *L. curvatus*, *L. plantarum*, *S. xylosus* and *L. fermentum* had two absorbance peaks at approximately 545 nm (β-band) and 580 nm (α-band) in the wavelength range of 500-600 nm (Fig. 2), which are the typical absorbance peaks of red myoglobin derivatives ($MbFe^{II}O_2$ and/or $MbFe^{II}NO$ (6). The control and samples treated with *P. pentosaceus* and *L. sake* showed absorption peaks at approximately 505 nm and 635 nm, which are characteristic of $MbFe^{III}$. The degree of $MbFe^{III}$ conversion to red derivatives varied depending on the bacterium strains inoculated, and *L. plantarum* could not convert $MbFe^{III}$ completely, as the two absorbance bands at 505 nm and 635 nm for $MbFe^{III}$ remained.

Millar et al. (6) reported that 544 and 582 nm are the maximal absorbance peaks for $MbFe^{II}O_2$,

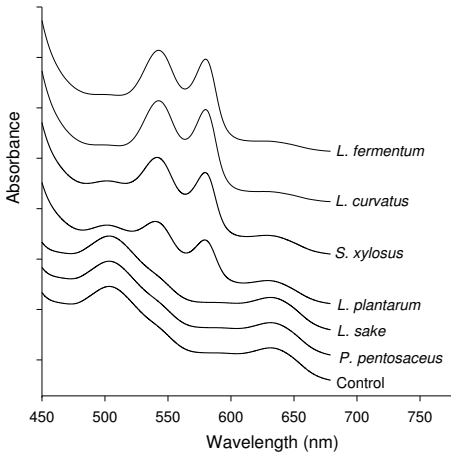


Fig. 2. Absorption spectra of $MbFe^{III}$-MRS broth inoculated with different bacteria strains.

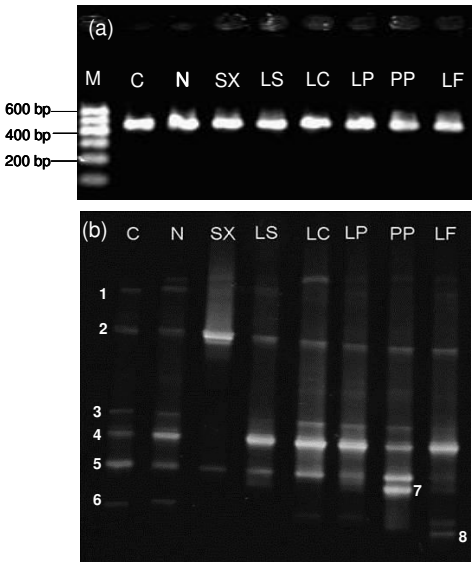


Fig. 3. 1.2% agarose electrophoresis profiles (a) of PCR products of bacterial DNA (V6~V8 region) extracted from different samples and their DGGE profiles (b).

whereas 548 and 579 nm are the maximal absorbance peaks for $MbFe^{II}NO$. Therefore, it is difficult to differentiate the UV-Vis absorption spectra between $MbFe^{II}O_2$ and $MbFe^{II}NO$ because the two red derivatives exhibit absorbance bands at similar wavelengths (approximately 545 and 580 nm). The absorbance between the β- and α-bands is one way to differentiate between the two derivatives because $MbFe^{II}NO$ has higher extinction coefficients at approximately 560 nm than $MbFe^{II}O_2$. However, such subtle differences become less useful when studying mixtures of the two absorbing species Therefore, the red pigment could be either $MbFe^{II}O_2$ or $MbFe^{II}NO$ or a mixture of the two. Nevertheless, it is interesting for meat colouring.

*3.2. DGGE analysis of bacteria diversity of raw meat batters*

The results obtained from the direct DGGE analysis of the bacterial community developed in meat batters with a denaturing gradient from 35% to 65% showed eight different profiles (Fig. 3b), which indicated that different microbial diversities were formed in the meat batters inoculated with different bacteria. The bands marked with different numbers in Fig. 3b were purified and then sequenced after re-amplification, and the relative identification obtained by alignment in GenBank is shown in Table 2.

The DGGE profiles of the control and nitrite-cured meat exhibited similar patterns (Fig. 3b). Bands of *Staphylococcus* spp., *Carnobacterium* sp., *Lactobacillus* sp., *Brochothrix thermosphacta* and *Enterococcus faecalis* were detected in the two treatments at the end of storage (day 12).

Table 2. Sequencing results from the bands cut from the bacterial DGGE gels shown in Fig. 3b

| Bands | Closest relative | % Identity |
|---|---|---|
| 1 | *Staphylococcus vitulinus* | 100 |
| 2 | *Staphylococcus xylosus* | 99 |
| 3 | *Carnobacterium* sp. | 100 |
| 4 | *Lactobacillus* sp. | 99 |
| 5 | *Pseudomonas* sp. | 97 |
| 6 | *Brochothrix thermosphacta* | 99 |
| 7 | *Pediococcus pentosaceus* | 99 |
| 8 | *Enterococcus faecalis* | 98 |

However, in this study, the DGGE profiles of meat batters inoculated with different LAB and *S. xylosus* bacteria were different between the control and nitrite samples. Only two bands (*S. xylosus* and *Pseudomonas* sp.) were detected in the sample inoculated with *S. xylosus*, which indicates that many spoilage bacteria were inhibited. Biological protection was also observed in the sample treated with *L. sake*, in which only bands of *S. xylosus*, *L. sake* and *Pseudomonas* sp. were observed. In

contrast, many other bands of spoilage microbes were observed in the meat batters inoculated with *L. curvatus*, *L. plantarum*, *L. fermentum* and *P. pentosaceus*.

*S. xylosus* has been reported to inhibit many unwanted bacteria, including *Listeria monocytogenes*in (7), *Bacillus licheniformis* (8), enterobacteriae and *Pseudomonas* (9), and the present study also demonstrates that many spoilage bacteria were inhibited by *S. xylosu*s. The antimicrobial role may be attributed to a bacteriocin (8, 9) or antagonistic substance (7) produced by *S. xylosus*. The role played by LAB in meat preservation is due to its strong ability to compete for growth nutrients, thrive at low pH and synthesis antimicrobial metabolites (10). Many studies have focussed on the effectiveness of *L. sake* as a protective culture against the growth of spoilage bacteria during refrigeration. Enterobacteriaceae, *Pseudomonas* spp., *B. thermosphacta* and *Leuconostoc mesenteroides* were observed to have been repressed in sliced beef at 4 °C (10) and in cooked ham at 4 or 7 °C (11, 12), respectively. Similar results were obtained in this study, in which most of spoilage bacteria were inhibited by *L. sake*.

## IV. CONCLUSION

Some lactic acid bacteria and *Staphylococcus xylosus* fermentation can perform a colouring and antimicrobial role in meat manufacturing. It may be developed as a potential method for nitrite substitution in meat products.

## ACKNOWLEDGEMENTS

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