Analysis of Potentially Antibacterial Components of Lactic Acid Bacteria against *Staphylococcus Aureus*

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Abstract: In our previous studies, *Lactobacillus plantarum* and *Lactococcus* genus, showed good antibacterial effects against *Staphylococcus aureus*, *Salmonella choleraesuis*, *Escherichia coli*, *Shigella*, *Salmonella*, *S. enteritidis*, and *S. enteritidis* subspecies isolated from the laboratory. However, antibacterial components of lactic acid bacteria against these key pathogenic bacteria such as *S. aureus* are not analyzed and speculated. In the current investigations, we tent to explore this question, and found that the active constituents of *S. aureus* were affected by the fermentation of lactic acid bacteria under different proteases and pH treatment. However, the bacteriostatic effect of the lactic acid bacteria fermentation liquid did not change significantly after heat treatment. It is speculated that the antibacterial active ingredient may be small peptides and organic acids in lactic acid bacteria, which has certain value for reducing the use of antibiotics and improving the quality and safety of pork foods.

1. Introduction

In recent years, the inhibitory effect of Lactic acid bacteria (LAB) on pathogenic bacteria in meat products has attracted wide attention of researchers, and the mechanism of inhibition has also been clarified [1-4]. In addition, in our previous study, antibacterial effects of lactic acid bacteria on several pathogenic bacteria were detected such as *S. aureus*, *Salmonella choleraesuis*, *Escherichia coli*, *Shigella*, *Salmonella*, *Salmonella enteritidis*, and *S. enteritidis* subspecies. However, antibacterial components of lactic acid bacteria against these key pathogenic bacteria such as *S. aureus* remain largely unknown. This study intends to analyze and speculate the potentially antibacterial substances.

2. Materials and methods

The lactic acid bacteria sterile fermentation broth was treated at 80 °C for 5 min, and the effect of the lactic acid bacteria sterile fermentation broth on the inhibition of *S. aureus* was detected by the Oxford cup double-layer plate method [5]. The untreated lactic acid bacteria sterile fermentation broth was used as a control.

Using the heat-treated fermentation broth as a control group, the MRS liquid medium was adjusted with lactic acid to the same liquid as the fermentation broth in experimental group, and the inhibition of the lactic acid bacteria sterile fermentation broth against *S. aureus* was measured by the Oxford cup double-layer plate method.

Different protease solutions were added to the lactic acid bacteria sterile fermentation solution at a final concentration of 1 mg/mL, and the enzyme was inactivated by a water bath at 37 °C for 2 h followed by at 80 °C for 2 min. The effect/inhibition of the sterile fermentation broth of lactic acid bacteria treated by different proteases on *S. aureus* was examined using the Oxford Cup double-layer plate method, as well as the sterile fermentation broth of untreated lactic acid bacteria was used as a control.

The cell-free culture solution of lactic acid bacteria with strong bacteriostatic effect was screened by ultraviolet spectrophotometer, and the absorbance value was measured at 600 nm. The OD value

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was adjusted to 1, and the supernatant was diluted 2, 4, and 8 times, respectively. The above dilutions were bacteriostatically tested against *S. aureus* by the Oxford Cup double-layer plate method. The minimum inhibitory concentration was determined by the concentration of the inhibition zone [6].

Table 1 Effects of different treatment methods on *S. aureus* bacteriostasis

Sample		Strain number (Diameter of inhibition zone (mm))			
		Lactobacillus		*Lactococcus*	
		PH 4.56	PH 6.5	PH 4.56	PH 6.5
CFS		28±0.23^{a}	18±0.15^{b}	30±0.36^{a}	20±0.25^{b}
Digestive enzyme treatment	Trypsin	28±0.26^{a}	9±0.12^{b}	29±0.28^{a}	0^{b}
	Pepsin	29±0.45^{a}	6±0.12^{b}	28±0.31^{a}	6±0.12^{b}
	Proteinase K	28±0.34^{a}	0^{b}	29±0.24^{a}	0^{b}
	Catalase				
Heat treatment	60℃	29±0.36^{a}		28±0.28^{a}	
	80℃	29±0.57^{a}		28±0.24^{a}	
	100℃	27±0.36^{a}		28±0.21^{a}	
2 times concentrated CFS		32±0.51^{a}		33±0.42^{a}	
Diluted	Diluted 2 times CFS	18±0.25^{b}		23±0.26^{a}	
	Diluted 4 times CFS	6±0.13^{a}		6±0.12^{a}	
	Diluted 8 times CFS	0^{a}		0^{a}	
MRS		0^{a}		0^{a}	

Note: Under the same parameters, the letters in the same row indicate significant differences ($p < 0.05$) and are repeated three times independently.

3. Results and discussion

The lactic acid bacteria inhibited the active components of *S. aureus* by heat treatment, lactic acid treatment, protease treatment and dilution treatment on two cell-free cultures of lactic acid bacteria. Table 1 shows effects of different treatments on *S. aureus* bacteriostasis. It can be seen that the bacteriostatic effect of the lactic acid bacteria changes in varying degrees after different treatments for the fermentation broth. After heat treatment at 80 °C for 5 min, the bacteriostatic ability of lactic acid bacteria had almost no effect, indicating that the thermal stability of the bacteriostatic substances was higher in the two lactic acid bacteria fermentation broth. After treatment with pepsin and trypsin, the bacteriostatic ability of the two strains of lactic acid bacteria decreased to varying degrees, indicating that there were proteins or peptides in the bacteriostatic substances in the two lactic acid bacteria fermentation broth. After the lactic acid bacteria fermentation broth was diluted, its antibacterial ability was greatly reduced. At the same time, the MRS medium with the same pH as the fermentation broth was used as the negative control, and the MRS liquid medium was used as the positive control, indicating that the bacteriostatic substances in the two lactic acid bacteria fermentation broth were organic acid.

The analysis of the active constituents of the two strains of lactic acid bacteria showed that these two strains had strong thermal stability, and showed some sensitivity to protease treatment. It is speculated that the active ingredient of lactic acid bacteria may be small peptides. In the experiment of determining the minimum inhibitory concentration for *S. aureus*, *L. plantarum* and *Lactococcus* showed a certain antibacterial effect on *S. aureus* after being diluted by 2 times, but the antibacterial ability of *Lactobacillus* disappeared after diluting 4 times. The results confirmed the maximum

dilution factor of these two strains of lactic acid bacteria against *S. aureus*. The lactic acid-treated MRS liquid medium showed a certain antibacterial effect, and it was speculated that the effective component of the bacteriostatic action of the lactic acid bacteria was an organic acid.

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