DECREASE OF *N*-NITROSODIMETHYLAMINE BY *LACTOBACILLUS PENTOSUS* IS ASSOCIATED WITH SURFACE LAYER PROTEINS

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Abstract –The objective of this study was to evaluate the ability of decreasing *N*-nitrosodimethylamine (NDMA) by five meat-borne bacteria species and to elucidate the mechanism in MRS broth. *Lactobacillus pentosus* was found to be the most effective in decreasing NDMA. It could not be reduced by either extracellular metabolites or intracellular extracts of *L. pentosus* ($p > 0.05$), and the proteins from the cell debris were found to be responsible for the decrease. The substances were considered as surface layer proteins which locate on the cell wall. It provided a potential way for decreasing NDMA in production of fermented meat products.

Key Words – lactic acid bacteria, *N*-nitrosamines, cell wall

I. INTRODUCTION

N-nitrosamines (NAs) in food have caused more and more concerns during the past decades, among which *N*-nitrosodimethylamine (NDMA) is recognized as probably carcinogenic to humans. Many ways have been attempted to decrease the NAs levels. A new approach is microbial decrease of NAs as a natural and sustainable method. A reduction of NDMA concentration was observed by *Lactobacillus* strains in MRS broth [1]. However, little is known about the mechanism. The objective of this study was to evaluate the ability of decreasing NDMA by five species belonging to meat-borne bacteria in MRS broth. Also, the component of the key ingredient and its location were identified to elucidate the mechanism.

II. MATERIALS AND METHODS

Five bacterial species tested were all isolated from Chinese dry sausage. Decreasing ability of NDMA was assessed according to Kim *et al*. [2]. The ability of the species was evaluated by the residual concentration of NDMA after cultivation in MRS broth. All tested species were inoculated in MRS broth containing 1 μg/mL NDMA. The control was prepared without inoculation.

Whole cells and cell-free supernatant (CFS) of *Lactobacillus pentosus* were prepared as described by Grill *et al*. [3]. The cells were washed with phosphate buffer solution (PBS), dissolved in PBS and the whole cell suspension (WCS) was obtained. The suspension was then subjected to ultrasonication and whole cell extracts (WCE) was gained. Intracellular extracts (IE) and cell debris were separated by centrifugation of the whole cell extracts. Cell debris was dissolved in PBS and cell debris suspension (CDS) was obtained. NDMA was added to CFS, WCS, WCE, IE and CDS to reach a final concentration of 1 μg/mL. NDMA concentration was determined after incubation.

Lipase, α-amylase and trypsin were used to determine the presence of lipid, glycogen and protein components in the key substances of decreasing NDMA in *L. pentosus*. Extraction of surface layer proteins (SLPs) and the SDS-PAGE analysis were performed according to the method of Meng *et al*. [4].

The data were analyzed using the SPSS package (Version 20, SPSS Inc., USA). Duncan's multiple range tests was used to compare the results of different treatments. The results were considered statistically significant at $p < 0.05$. Each experiment was performed in triplicate.

III. RESULTS AND DISCUSSION

The five species all isolated from one meat system were observed to exhibit different abilities in decreasing NDMA (Fig. 1), which was in agreement with Kim *et al*. [2]. Among them, *L. pentosus* was found to be most effective, with a decreasing rate of 22.05%.

CFS of *L. pentosus* was found to have no effect on NDMA compared with the control ($p > 0.05$, Fig. 2), but the WCS could decrease it significantly ($p < 0.05$). It turned out that NDMA was decreased via meta-

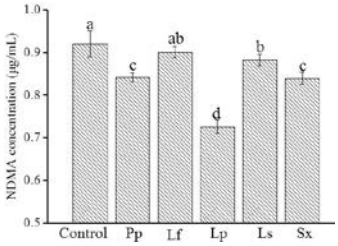

Figure 1. Decreasing NDMA by different species in MRS.

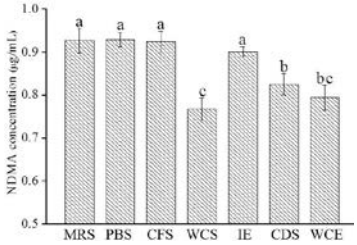

Figure 2. Decreasing NDMA by different cell components

bolism of the *L. pentosus* cells. For the different cell components, the CDS showed decreasing ability of NDMA but the IE did not, which indicates the active components involved in NDMA decreasing were located on the cell membrane or cell wall.

The effective components of decreasing NDMA were not sensitive to α-amylase and lipase (Fig. 3). However, when treated with trypsin, the decreasing rate became significantly lower ($p < 0.05$), which means that proteins located on cell membrane or cell wall of *L. pentosus* may play an important role. SDS-PAGE was employed to analyses the proteins extracted using 5 mol/L LiCl, and a dominant protein band with a MW of 45-60 kDa was found only in the *L. pentosus* sample, which confirmed the presence of SLPs in the strain. SLPs may help the bacterial cells to adhere NDMA and provide attachment sites for the degradation enzymes, or they may be a series of special enzyme systems that can degrade the NAs [4].

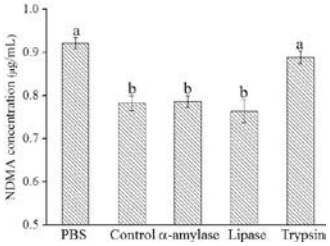

Figure 3. Effects of enzyme treatments on NDMA concentration.

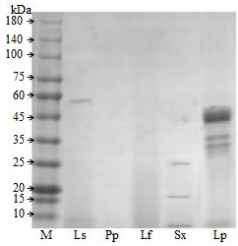

Figure 4. SDS-PAGE analysis of SLPs.

IV. CONCLUSION

L. pentosus was found to be the most effective in decreasing NDMA, and the proteinaceous substances in the cell debris were observed to be responsible for the decrease.

ACKNOWLEDGEMENTS

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