EFFECT OF PROBIOTIC *BACILLUS CEREUS* DM423 ON THE FLAVOR FORMATION OF FERMENTED SAUSAGE

Kai Shan[1], Chunbao Li[1,*]

[1] Key Laboratory of Meat Processing and Quality Control, MOE; Key Laboratory of Meat Processing, MARA; Jiangsu Synergetic Innovation Center of Meat Processing and Quality Control; College of Food Science and Technology, Nanjing Agricultural University, Nanjing, Jiangsu 210095, China

*Corresponding author email: chunbao.li@njau.edu.cn.

I. INTRODUCTION

The poor flavor of lactic acid bacteria (LAB) fermented sausage can be caused by the insufficient protein and fat hydrolysis capacity of LAB [1-2]. Therefore, using more bacteria as co-starters in fermented sausage could be a promising research direction. *Bacillus* is a well-established provider of protease and lipase, and some probiotic strains have been used in food processing [3]. Although *B. cereus* is often regarded as a kind of saprophytic or pathogenic microorganism, there are also many safe and functional strains which have been used as human probiotics such as *B. cereus* IP 5832 in Bactisubtil®. The probiotic functions of *B. cereus* are often originated from the ability of fibre degradation as well as expression of proteases, lipases, nucleases, and phosphatases implying that *B. cereus* may have a strong ability to metabolize meat components [4]. Therefore, in this study, the effect of a strain of probiotic *B. cereus* (DM423) in sausage fermentation was explored.

II. MATERIALS AND METHODS

Food grade *L. plantarum* HH-LP56 and *L. rhamnosus* were cultured in MRS medium at 30 °C. *B. cereus* DM423 was from YUANSHOUKANG® live *B. cereus* capsule (S10980014, Yuanshou Biological Pharmaceutical Co., Ltd., Anyang, China) and cultured in tryptone soya broth at 30 °C. The biogenic amines forming capacity was evaluated in Niven's medium. The flavor was evaluated by e-nose, e-tongue, and GC-IMS. The lipid profile was observed by thin-layer chromatography and the protein hydrolysis was evaluated by fluoroamine. Next-generation sequencing was used for genome *de novo* sequencing. One-way ANOVA with Bonferroni correction was performed to analyze statistical differences between multiple groups.

III. RESULTS AND DISCUSSION

The biosafety of DM423 has been confirmed before its clinical application. However, its ability to produce biogenic amines was still worth evaluating. In Niven's medium, *L. plantarum* HH-LP56 and *L. rhamnosus* GG turned the solution yellow indicating the biogenic amines production, but DM423 did not have this effects (Fig. 1A). By an e-tongue, we found that *B. cereus* enhanced saltiness, umami, and aftertaste-B. It also attenuated sourness, bitterness, and astringency (Fig. 1B). Quantitatively, DM423 up-regulated free amino acid content (Fig. 2C). By an e-nose, we found that *B. cereus* enhanced the signal of W1C, W3C and W1S while attenuated the signal of W2W, W1W, and W6S before heating (Fig. 1D). Through thin layer chromatography, we found that *B. cereus* did not intensely reduce TAGs but promoted further degradation of diglycerides and free fatty acids (Fig. 1E). Through GC-IMS, it can be observed that DM423 caused obvious changes in volatile composition (Fig. 1F). To understand the mechanisms by which B. cereus promoted proteolysis, lipolysis, and flavor formation, the genomes of *B. cereus* DM423 and *L. plantarum* HH-LP56 were sequenced. Among conventional starters *(Lactobacillaceae, Streptococcaceae,* and *Staphylococcaceae*) we aligned DM423 enriched peptidase, protease, lipase, and unique flavor-associated genes with previously reported sequences. When comparing the proportion of homologous genes in individual bacteria to all genes that have been identified, we found that the enriched genes in DM423 (compared to *L. plantarum* HH-LP56) were also

widely distributed in the *Bacillaceae*. Meanwhile, these genes were still very rare in *Lactobacillaceae, Streptococcaceae,* or *Staphylococcaceae* (Fig. 1G). This implied tha, not only DM423, other *Bacillus* species can effectively complement the metabolic defects of conventional fermentative species.

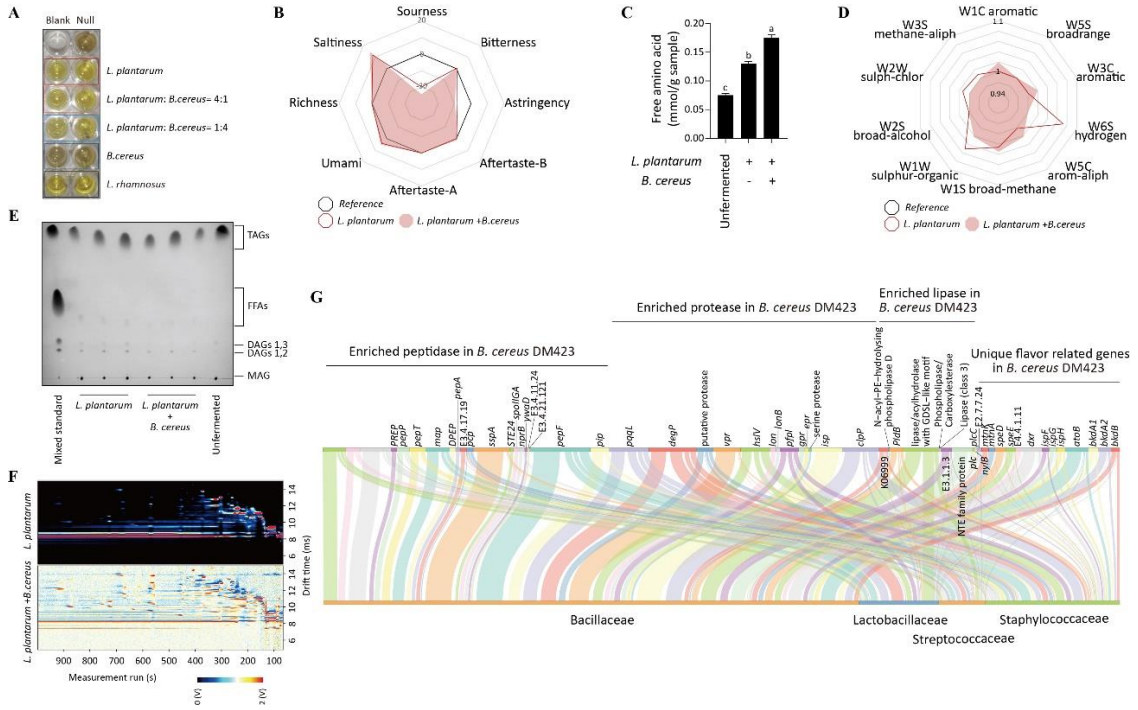

Figure 1. Effect of probiotic *Bacillus cereus* DM423 on the flavor formation of fermented sausage

IV. CONCLUSION

In this study, a strain of probiotic *Bacillus cereus* was used as a co-starter to improve the flavor of fermented sausage, and this was due to the abundance of metabolic enzymes derived from *Bacillus cereus*. Through genomic analysis, we confirmed that *Bacillus* has a general advantage in flavor production over LAB and *Staphylococcus* which are traditionally used in meat fermentation. Using *B. cereus* as a model, this study sought to elucidate a molecular biology information-based strategy for the screening of bacteria for fermentation: probiotics that can form a functional supplement to the genome of LAB can be considered candidate co-starters. Although the safety of this strategy needs further validation, it is expected to greatly accelerate the research on novel starters.

ACKNOWLEDGEMENTS

This research is supported by the Ministry of Finance and the Ministry of Agriculture and Rural Affairs of China (Cars-35), Jiangsu Innovation Group of Meat Nutrition, Health and Biotechnology and Jiangsu College Student Innovation and Entrepreneurship Training Program (202210307198Y).

REFERENCES

1. Javed, S., Azeem, F., Hussain, S., Rasul, I., Siddique, M. H., Riaz, M., Afzal, M., Kouser, A., & Nadeem, H. (2018). Bacterial lipases: A review on purification and characterization. Progress in biophysics and molecular biology 132: 23–34.
2. Lin L., Yanshun X. (2021). Influence of Lactobacillus plantarum on managing lipolysis and flavor generation of Staphylococcus xylosus and Saccharomyces cerevisiae in fish paste. Lebensmittel-Wissenschaft und-Technologie 140: 110709.
3. Contesini, F. J., Melo, R. R., & Sato, H. H. (2018). An overview of Bacillus proteases: from production to application Critical reviews in biotechnology 38: 321–334.
4. Lee, N. K., Kim, W. S., & Paik, H. D. (2019). Bacillus strains as human probiotics: characterization, safety, microbiome, and probiotic carrier. Food science and biotechnology 28: 1297–1305.