SEARCHING FOR HIGH ZnPP-FORMING BACTERIA FOR APPLICATION TO MEAT PRODUCTS

M. Asaduzzaman, M. Ohya, M. Akter, T. Hayakawa, H. Kumura, J. Wakamatsu

Applied Food Science Laboratory, Division of Bio-systems Sustainability, Graduate School of Agriculture,

Hokkaido University, N-9, W-9, Kita-ku, Sapporo, Hokkaido 060-8589, Japan

*Corresponding author email: jwaka@anim.agr.hokudai.ac.jp

I. INTRODUCTION

Color of meat products is an important quality attribute influencing the consumer's purchase decision. In general nitrate/nitrite is added to cured meat products as coloring/preservative agent. But there is a possibility that nitrite reacts with secondary amines to form nitrosamines, a potent carcinogen. For this reason, many consumers desire nitrate/nitrite-free meat products. On the other hand, Parma ham has a characteristic red color without nitrate/nitrite. The bright red color of Parma ham derived from zinc protoporphyrin IX (ZnPP) [1]. Microorganisms were suggested not to contribute to the formation of ZnPP in Parma ham and the amount of ZnPP was increased in pork homogenate without addition of antibiotics [2]. However, it has been reported that some Staphylococci isolated from Parma ham have a capability to produce ZnPP [3]. Accordingly, some edible bacteria might be useful to improve the color of meat products by producing ZnPP. Therefore, this study was undertaken to search for edible bacteria that can produce ZnPP in meat product as a substitute of nitrate/nitrite.

II. MATERIALS AND METHODS

High ZnPP-forming bacteria were isolated from two different categories of sources, (1) bacteria from animal food products (11 species), *Carnobacterium divergens* and *Serratia proteamaculans* were used as known source of producing ZnPP [4] and (2) bacteria from various environmental sources (Bacteria were grown at pH 5.5 with 3% salt in the standard plate count agar at 20-25^{o}C under anaerobic condition).

a. Meat homogenate model system

High ZnPP-forming ability of both sources of bacteria was evaluated in meat homogenate model system. Sterile meat homogenate (20%) containing 3% salt received test strain (2.0 ×10^{6}/ml) to incubate 25^{o}C for 7 and 14 days under anaerobic condition. Finally, ZnPP was extracted with 75% acetone and measured the fluorescence intensity at ex/em 420/590nm.

b. Meat model experiment

Meat model experiment was performed to check normal color and ZnPP formation by selected bacteria. Sterile minced meat was prepared by *longissimus* muscle with 3% salt. Nine grams of minced meat and 1 ml of bacterial culture (2×10^{6}/ml) were mixed and vacuum packed. Incubation was done at 18^{o}C for 7 and 14 days. The surface autofluorescence was observed as index of ZnPP-formation.

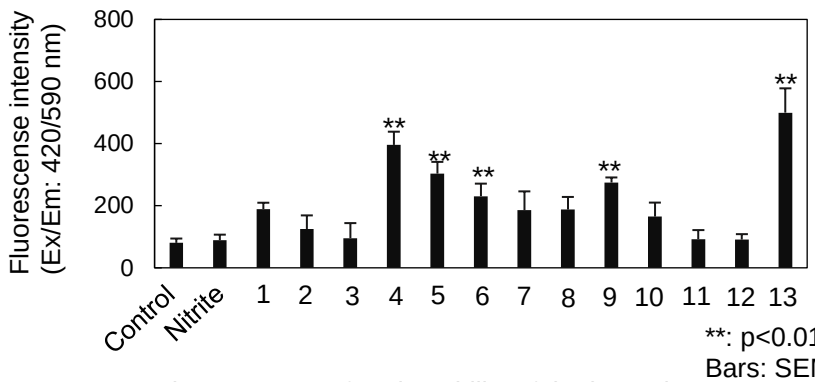
III. RESULTS AND DISCUSSION

a. ZnPP-forming ability of bacteria from animal food product source

As shown in Fig.1, it was observed that *L. plantarum, L. rhamnosus, L. sakei, K. varians* and *S. proteamaculans* produced significantly higher amount of ZnPP after 7 days of incubation. *C. divergens* could not produce ZnPP because of the high salt content of the homogenate. On the other hand, since there was no difference between the non-added group (control) and the nitrite group, it was considered that only the inoculated microorganisms were involved in the ZnPP-formation in meat homogenate.

b. Isolation and identification of high ZnPP-forming bacteria from environmental source

We obtained 44 colonies from various environmental sources as high ZnPP-forming bacteria on meat homogenate model system. Fourteen species (avoid duplication) were identified by 16S rRNA full length sequencing. We successfully isolated three edible strain to be potential for meat product application.



Inoculated bacteria
1. *Lactobacillus brevis*
2. *Lactobacillus buchneri*
3. *Lactobacillus curvatus*
4. *Lactobacillus plantarum*
5. *Lactobacillus rhamnosus*
6. *Lactobacillus sakei*
7. *Pediococcus acidilactici*
8. *Pediococcus pentosaceus*
9. *Kocuria varians*
10. *Shaphylococcus carnosus*
11. *Shaphylococcus xylosus*
12. *Carnobacterium divergens*
13. *Serratia proteamaculans*

Figure 1. ZnPP-forming ability of the bacteria after 7 days of incubation

c. Color and ZnPP-forming ability in meat by selected bacteria

Among the microorganisms added to minced pork, *S. proteamaculans, L. plantarum, L. mesenteroides* and *L. lactis* groups have a capability to produce brighter red color compared with the control and showed similar color with that of the nitrite group (Fig. 2). Regarding the ZnPP autofluorescence, there is no fluorescence in the nitrite group (Fig. 2). Among the microorganisms, autofluorescence in *S. proteamaculans* group was the strongest. The ZnPP autofluorescence of *L. mesenteroides, L. lactis* and *L. plantarum* groups were higher compared with the other bacteria groups.

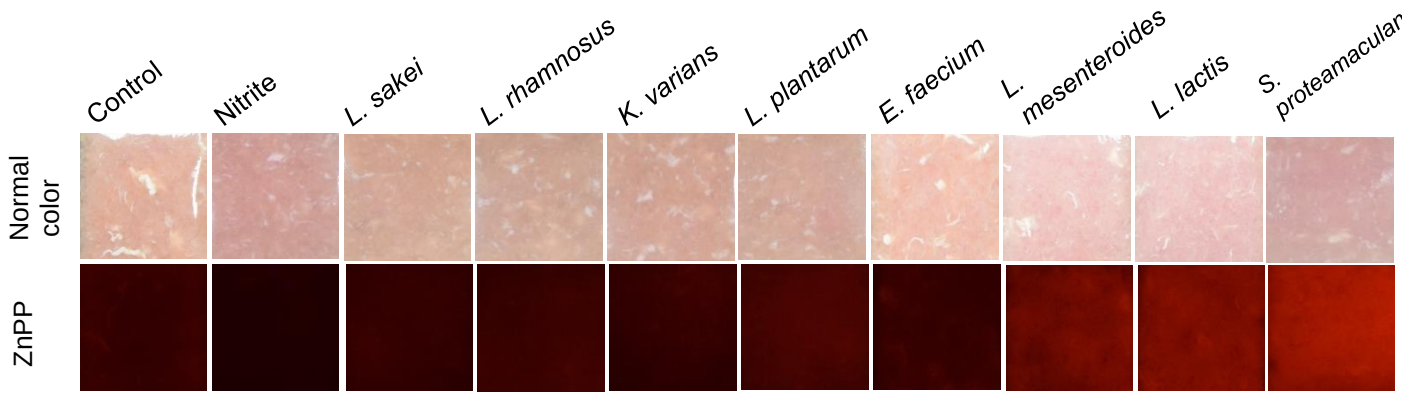


Figure 2. Normal color and ZnPP autofluorescence of minced meat after 14 days of incubation

IV. CONCLUSION

The results in this study suggested that some edible bacteria have the high potential to produce ZnPP in meat products. These bacteria would be the promising candidate to improve color of cured meat products as substitute of nitrate/nitrite.

REFERENCES

1. Wakamatsu, J., Nishimura, T. & Hattori, A. (2004a). A Zn-porphyrin complex contributes to bright red color in Parma ham. Meat Science 67: 95-100.
2. Wakamatsu, J., Okui, J., Ikeda, Y., Nishimura, T. & Hattori, A. (2004b). Establishment of a model experiment system to elucidate the mechanism by which Zn–protoporphyrin IX is formed in nitrite-free dry-cured ham. Meat Science 68: 313-317.
3. Morita, H., Niu, J., Sakata, R. & Nagata, Y. (1996). Red pigment of Parma ham and bacterial influence on its formation. Journal of Food Science 61: 1021-1023.
4. Kawazoe, H., Shiraishi, A., Parolari, G., Nishimura, T. & Wakamatsu, J. (2012). Effects of acidifiers, pH and microorganisms on the formation of zinc protoporphyrin IX in pork homogenate. In proceedings 58th International Congress of Meat Science and Technology, 12-17 August 2012, Montreal, Canada.