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Isolation of bacteria having potentiality to form ZnPP in meat products (#52)

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Introduction

Zinc protoporphyrin IX (ZnPP) is a main natural red pigment found in Parma ham without nitrate/nitrite (Wakamatsu *et al.* 2004a). ZnPP is thought to be a promising pigment to improve the color of meat products without nitrate/nitrite. Microorganisms had been considered not to contribute to the formation of ZnPP in Parma ham. However, some staphylococcal strains isolated from Parma ham have a capability to produce ZnPP (Morita *et al.* 1996). Furthermore, the amount of ZnPP was increased in pork homogenate without the addition of antibiotics (Wakamatsu *et al.* 2004b). Accordingly, some edible bacteria might have potential for producing ZnPP, which would be advantageous to improve the color of meat products. Therefore, the aim of this study was to screen suitable bacteria that can improve the color of meat products with the production of ZnPP under the nitrate/nitrite–free conditions.

Methods

a) Isolation and identification of high ZnPP–forming bacteria

Bacteria from various sources were cultured on the modified standard plate count agar containing 3% NaCl with adjusted at pH 5.5 because of application of making fermented sausage. Bacteria were incubated at 20–25°C in anaerobic condition. ZnPP–forming ability of isolated bacteria were evaluated in the established meat homogenate model system (Wakamatsu *et al.* 2004b). High ZnPP–forming bacteria were identified by 16S rRNA full–length sequencing.

b) Evaluation of ZnPP–forming ability of inoculated bacteria in aseptic minced meat and dry fermented sausage

Aseptic minced pork was prepared from *longissimus* muscle, mixed with 3% salt and the isolated edible bacteria. After vacuum packing it was incubated at 18°C for 14 days. Dry fermented sausage was prepared by adding identified edible bacteria. Fluorescence intensity (Ex/Em: 420/590 nm) of ZnPP was measured by 75% acetone extraction method. Autofluorescence of ZnPP in minced pork and fermented sausages was observed with purple LED lights and band pass sheet filter–equipped digital camera (Wakamatsu *et al.* 2009).

Results

a) Isolation and identification of high ZnPP–forming bacteria

As shown in Fig. 1, fluorescence intensity of meat homogenates was depending on the inoculated bacteria. Some of them showed significantly higher intensity than control. Since there was no difference in ZnPP production between control and antibiotic groups, it was obvious that the meat homogenate was prepared aseptically and the increased ZnPP was ascribed to the action of inoculated bacteria. Finally, 44 colonies having high ZnPP–forming ability were isolated. Seventeen species of bacteria were identified by 16s rRNA full–length gene sequencing and out of them 3 bacteria (X, Y and Z) were found to be edible.

b) ZnPP–forming ability of inoculated bacteria in aseptic minced meat and dry fermented sausage

Fig. 2A showed brighter red color and strong ZnPP autofluorescence in the samples containing bacteria X and Y compared to control in which bacteria was not inoculated. Although the aseptic minced meat, receiving bacteria Z showed comparable ZnPP autofluorescence to the control, fluorescence intensity of ZnPP in the all three inoculated groups were significantly higher than that of control (Fig. 2B). When the identified edible bacteria were applied for manufacturing of sausages, brighter red color was observed in the inoculated and nitrite–added sausages compared to control (Fig. 3A). Strong ZnPP autofluorescence was observed in the inoculated groups whereas negligible autofluorescence was observed in nitrite group. Fluorescence intensity of ZnPP was also significantly higher in the inoculated sausages compared to nitrite and control groups (Fig. 3B). The inoculated edible bacteria (especially X and Y) showed strong capability to form ZnPP in meat system and sausages.

Conclusion

These identified edible bacteria havethe high potential to produce ZnPP in meat products. These high ZnPP–forming bacteria can be the candidates to improve the color of meat products without nitrate/nitrite.

Notes

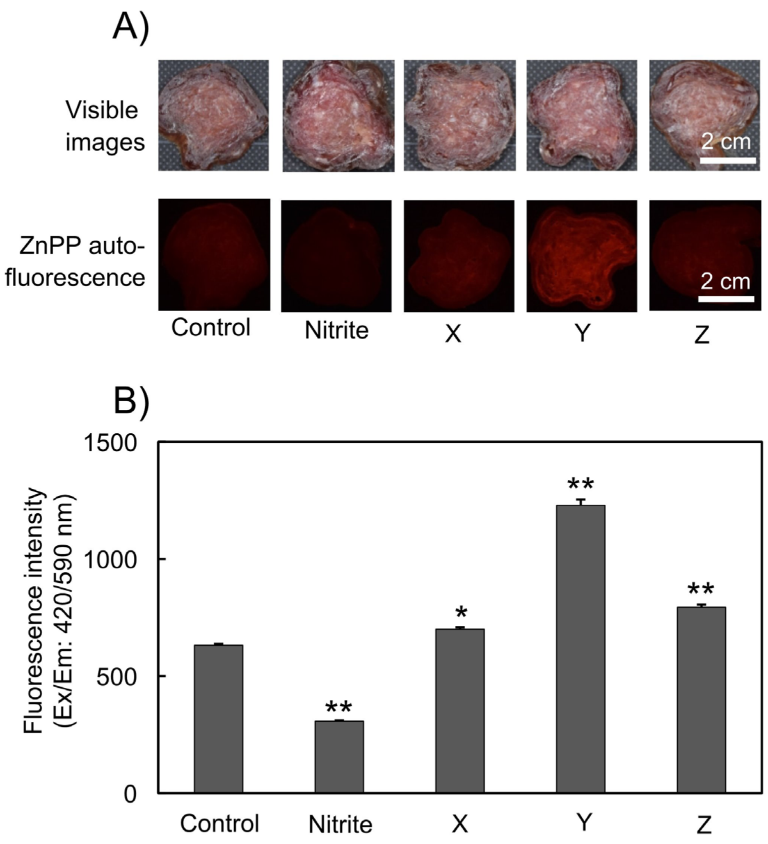

Fig. 3. ZnPP-forming ability of the isolated edible bacteria in fermented sausages
A) Visible images and ZnPP autofluorescence of sausages with the high ZnPP-forming edible bacteria. B) Fluorescence intensity of ZnPP (measured by acetone extraction method) in the fermented sausages.* and **: $P < 0.05$ and $P < 0.01$ vs. control; Bars: SEM ($n = 3$)

Notes

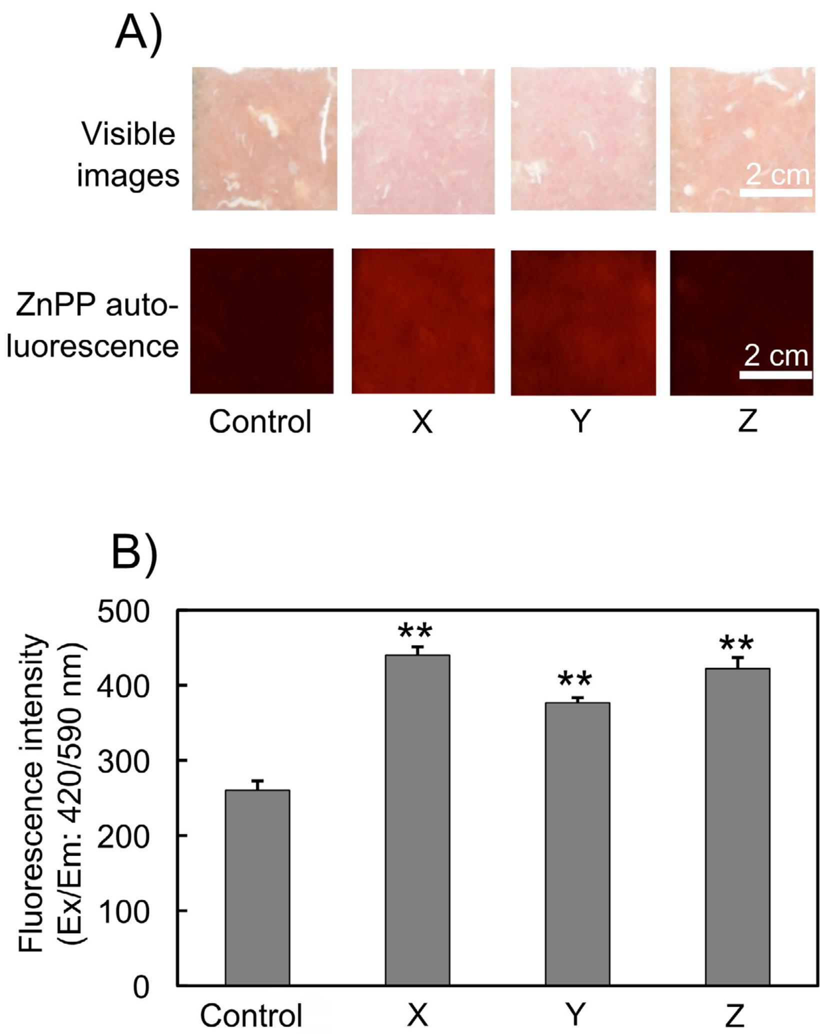

Fig. 2. ZnPP-forming ability of the isolated edible bacteria in aseptic minced meat
A) Visible images and ZnPP autofluorescence of aseptic minced meat with the high ZnPP-forming edible bacteria X, Y and Z.
B) Fluorescence intensity of ZnPP (measured by acetone extraction method) in the minced meat.**: $P < 0.01$ vs. control;
Bars: SEM ($n = 2$)

Notes

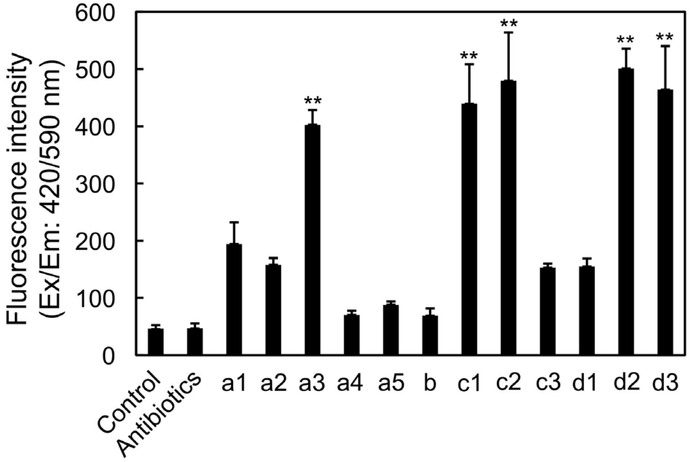

Fig. 1. Evaluation of ZnPP-forming capability of isolated bacteria in meat homogenate model system

Aseptic 20% meat homogenate was prepared with 3% salt and bacteria (2×10^6/ml) was inoculated, after incubation at 25ºC for 7 days in ana-erobic condition, fluorescence intensity of ZnPP was measured by acetone extraction method. Non-inoculated group designated as con-trol. a, b, c and d indicates the various sources of bacteria and 1, 2, 3, 4 and 5 indicates different isolate in each source. **: $P < 0.01$ vs. control; Bars: SEM (n = 3)

Notes