CONVERSION OF METMYOGLOBIN TO BRIGHT RED DERIVATIVES BY LACTIC ACID BACTERIA

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SUMMARY

A total of 152 characterized lactic acid bacteria (90 *Lactobacillus*, 28 *Enterococcus*, 21 *Pediococcus*, 8 *Lactococcus*, 4 *Streptococcus*, 1 *Leuconostoc*) and 358 uncharacterized lactic acid bacteria were screened for metmyoglobin conversion activity. Agar plates containing metmyoglobin were inoculated with the test organisms and subsequently examined for color changes around colonies. Of the 510 strains tested, 6 strains (1 *Lactobacillus fermentum* JCM1173, 3 *Enterococcus faecalis*, 1 *E. gallinarum*, and 1 *E. mundtii*) consistently converted metmyoglobin to bright red myoglobin derivatives. These 6 strains also converted metmyoglobin to a more desirable derivative in broth. The myoglobin derivative formed by the enterococci had a visual spectrum typical of oxymyoglobin, whereas *Lactobacillus fermentum* JCM1173 generated nitric oxide myoglobin. Collectively, these data indicate that bacterial conversion system(s) may find utility for improving the appearance of meat products during storage.

Introduction

Meat color is largely dependent on the chemical state of myoglobin, a heme-containing protein in muscle. Fresh meat color is attributed to oxymyoglobin; however, oxymyoglobin is not stable during storage or heating. Undesirable discoloration on the surface of meat during storage is due to the accumulation of metmyoglobin (oxidized form), which is brown. Formation of metmyoglobin is also associated with lipid oxidation in meat (Faustman et al., 1989). Preventing metmyoglobin accumulation is one approach for preserving the salable quality of meats.

In addition to the oxidation reaction, myoglobin reacts with nitric oxide (from nitrite) to generate other typical color components (e.g., nitric oxide myoglobin) during curing (Cassens et al., 1979). The characteristic pink color of certain meats is contributed by the transformation of nitric oxide myoglobin to nitrosyl myoglobin upon heating. Unfortunately, nitrite has serious disadvantages including formation of carcinogenic N-nitrosamines in some cured products or in the stomach. For these reasons, there is an increased demand for nitrite-free meat products and an intensified effort to develop a substitute for nitrite to cure meats.

Although bacteria can influence meat color, until recently, attempts have not been made to exploit bacterial conversion of myoglobin to more desirable color derivatives for preserving or improving meat products. In this study, lactic acid bacteria were screened for the ability to convert metmyoglobin to bright red derivatives. Experiments were also conducted to characterize the myoglobin derivatives generated by such bacteria.

Materials and methods

The materials and methods used in this study are described in detail elsewhere (Arihara et al., 1993a; 1994a). Therefore, only the major experimental procedures are outlined herein.

Metmyoglobin conversion activity was assayed for 2 bacterial groupings of lactic acid bacteria (LAB) strains. Group A consisted of 347 LAB [81 characterized strains (66 *Lactobacillus*, 15 *Pediococcus*, 1 *Leuconostoc*) from several culture repositories and 266 uncharacterized environmental isolates; Arihara et al., 1993a]. Group B consisted of 163 LAB [71 characterized strains (24 *Lactobacillus*, 8 *Lactococcus*, 4 *Streptococcus*, 28 *Enterococcus*, 7 *Pediococcus*) from our collection and 92 uncharacterized environmental isolates; Arihara et al., 1993b; 1994a].

Figures 1 and 2 show the procedures of screening bacteria for metmyoglobin conversion activity and characterization of myoglobin derivatives generated by LAB, respectively.

Results and discussion

Conversion of metmyoglobin by group A isolates

Of 347 diverse lactic acid bacteria tested (group A), only one strain, *Lactobacillus fermentum* JCM1173 generated a bright red myoglobin derivative on agar plates containing metmyoglobin. In broth media containing metmyoglobin, overnight incubation of strain JCM1173 also generated a bright red color. The visible spectrum of this derivative could not be measured due to the insoluble state of myoglobin, presumably caused by acid denaturation of the globin moiety of myoglobin (i.e., due to production of lactic acid by JCM1173). However, metmyoglobin solution in dialysis tubing changed from a brown to bright red color when suspended in MRS broth containing 0.2% glucose in the presence of *L. fermentum* JCM1173 (Figure 3). The concentration of glucose in MRS was reduced from 2% to 0.2% to prevent acid denaturation of myoglobin. During the initial growth phase of JCM1173, it was possible to observe spectral changes leading to formation of a myoglobin derivative (Figure 4). The visible spectrum of the derivative generated by JCM1173 was similar to that of nitric oxide myoglobin. Furthermore, the visible spectrum of the heme-moiety solution extracted from the myoglobin derivative generated by strain JCM1173 was similar to the spectrum of nitric oxide myoglobin (data not shown).

Nitric oxide formation by *Lactobacillus* sp. is desirable in meat technology, since this intermediate is required in the reddening reaction. In cured meats, nitric oxide myoglobin is formed by the reaction of myoglobin with nitric oxide generated from nitrite (Kamarei and Karel, 1982). Although Wolf et al. (1990) reported that some lactobacilli reduced nitrite to nitric oxide, nitrite was not used in our assays and sufficient nitrite is not available in MRS broth to support nitric oxide production. It is uncertain how *L. fermentum* JCM1173 formed nitric oxide myoglobin without addition of nitrite to the media. In another mechanism for generating nitric oxide that is not dependent on nitrite, some mammals produce nitric oxide using L-arginine as a substrate (Stuehr et al., 1990). However, to date, nitric oxide synthase that converts L-arginine to nitric oxide has not been found in bacterial cells.

L. fermentum JCM1173 is of particular interest for application in meat systems, because lactobacilli are widely used as starter cultures in meat fermentations, where they contribute to flavor development and product safety.

Conversion of metmyoglobin by group B isolates

Of 163 LAB strains tested (group B), 5 *Enterococcus* isolates (*E. faecalis* JBL1048, JBL1055, JBL1057, *E. gallinarum* JBL1046, *E. mundtii* JBL1069) consistently converted metmyoglobin to bright red derivatives on agar plates. These 5 strains also converted metmyoglobin to a more desirable derivative in broth. As shown in Figure 5, spectral analysis of the myoglobin derivative generated in liquid media by these strains revealed identity with oxymyoglobin. With the exception of JBL1069, generation of oxymyoglobin was accomplished within 2.5 h (Figure 5).

A decrease in the amount of metmyoglobin by aerobic bacteria or yeasts in meat has been reported, but the myoglobin derivatives generated were not identified (Roback and Costilow, 1961; Faustman et al., 1990). In contrast, Arihara et al. (1993a) recovered *Kurthia* sp. that reduced metmyoglobin and identified the derivatives as oxymyoglobin. As one explanation, aerobic bacteria consume oxygen and create an environment low in oxygen pressure that allows for metmyoglobin reduction. However, enterococci are facultative anaerobes, and as such oxygen is not required for growth (Wittenbury, 1978). Although some strains of LAB, including enterococci, consume oxygen via flavoprotein oxidase systems and produce H_2O_2, detectable levels of H_2O_2 were not found in liquid media during incubation of the 5 *Enterococcus* strains (data not shown). Moreover, as detailed in Figure 5 and Table 1, metmyoglobin conversion to oxymyoglobin was completed within 2.5 h without sufficient growth of the enterococci. For these reasons, it seems likely that metmyoglobin reduction by enterococci was not due to bacterial oxygen consumption.

Neither culture filtrates nor washed cells of strains JBL1046, 1048, 1055, 1057 or 1069 exhibit metmyoglobin conversion activity (data not shown). These data indicate that either metmyoglobin conversion occurs intracellularly or that several components are necessary for extracellular conversion activity. Regarding the latter hypothesis, it is possible that a cell-bound enzyme, perhaps in concert with a co-factor, converted metmyoglobin to oxymyoglobin by the enterococci identified herein. A cell-bound metmyoglobin reducing

enzyme system has been found in muscle tissue (Arihara et al., 1994b). Further investigation is required to elucidate the mechanism of bacterial metmyoglobin reduction activity by the 5 enterococci identified in this study.

Conclusion

This study identified LAB capable of converting metmyoglobin (brown in color) to more desirable myoglobin derivatives (nitric oxide myoglobin and oxymyoglobin; bright red in color). These data establish the potential that metmyoglobin conversion systems by LAB may be used to preserve or improve the appearance of meat products.

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