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The basis of nitrosomyoglobin formation in meat by coagulase-negative staphylococci: Expression of nitric oxide synthase (#356)

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Introduction

Color is essential for fermented meat products in which nitrite addition is responsible for the development of the characteristic red color. However, toxic *N*-nitrosamines may form from nitrite, which has raised public concerns. Nitric oxide synthase (NOS) pathway in coagulase-negative staphylococci (CNS) is regarded as the main source of nitric oxide (NO) [1], which can form nitrosomyoglobin (NO-Mb) instead of relying on nitrite. Although the *nos* gene has been detected in CNS [2], *nos* gene expression is not clarified. The objective of this study was to demonstrate *nos* gene expression in three CNS species and to assess their abilities to produce NO.

Methods

Three CNS species were tested in this study. *Staphylococcus carnosus* GIM 1.955 was obtained from China Guangdong Institute of Microbiology (Guangdong, China). *Staphylococcus equorum* E1 was isolated from Tongcheng dry-cured duck.*Staphylococcus vitulinus* CICC 10850 was obtained from China Center of Industrial Culture Collection (Beijing, China).

Total DNA and RNA of the three CNS species were extracted from the cells using TIANamp Bacteria DNA Kit (Tiangen, Beijing, China) and Spin Column Bacteria Total RNA Purification Kit (Sangon Biotech, Shanghai, China), respectively, following the manufacturer's protocol. cDNA synthesis from total RNA was performed using the FastQuant RT Kit (Tiangen) according to the manufacturer's protocol. The PCRs were performed in a T100 Thermal Cycler (Bio-Rad, USA) with different primer pairs. All primer pairs were designed using Primer 3 software. The primers of *S. carnosus*, *S. equorum* and *S. vitulinus* were designed according to the ENA accession no. of the entries NC_012121.1, NZ_CP013114.1 and NZ_QXTO01000059.1, respectively. Western blot experiment was conducted with an anti-rabbit iNOS antibody (Bioss, Beijing, China).

Results

A. Evidence for *nos* gene expression

The PCR and reverse transcriptional PCR showed that the DNA of the three CNS species could be transcribed to mRNA successfully, since the bands of the expected sizes were found in Fig. 1A and Fig. 1B. As shown in Fig. 1C, the NOS protein was found to exist in all of the three species of CNS by western blot analysis. Thus, it was demonstrated that the three CNS species possessed a *nos* gene that could be transcribed to mRNA and then translated into protein successfully, which was the basis of nitric oxide production.

B. Phenotype of NO production

NO production was detected by the NO-specific, fluorescent DAF-FM DA probe. Green fluorescence was observed in the three CNS cells (Fig. 2), suggesting that NO was produced by the three CNS species. Together with the gene results, the phenotype of NOS was observed in the three CNS species. Thus, it is indicated that NO-Mb could be formed in meat without nitrite addition.

Conclusion

The expression of *nos* gene in CNS was systematically proven from DNA to RNA to protein, and NO generation was also directly detected. This work provides a basis of NO-Mb formation in meat products without nitrite addition.

References

1. Ras, G., Bailly, X., Chacornac, J. P., Zuliani, V., Derkx, P., Seibert, T. M., Talon, R. & Leroy S. (2018). Contribution of nitric oxide synthase from coagulase-negative staphylococci to the development of red myoglobin derivatives. International Journal of Food Microbiology 266: 310-316.
2. Sánchez-Mainar, M., Weckx, S. & Leroy, F. (2014). Coagulase-negative Staphylococci favor conversion of arginine into ornithine despite a widespread genetic potential for nitric oxide synthase activity. Applied and Environmental Microbiology 80: 7741-7751.

Notes

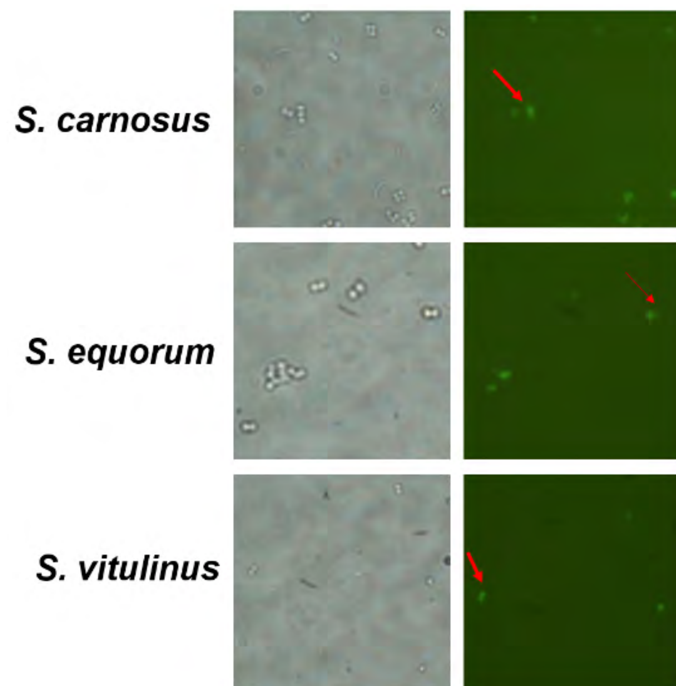

Fig. 2. NO production in three CNS cells.
Cell morphology was observed in brightfield (left panels), and NO production was shown as fluorescence from DAF-FM DA (right panels).

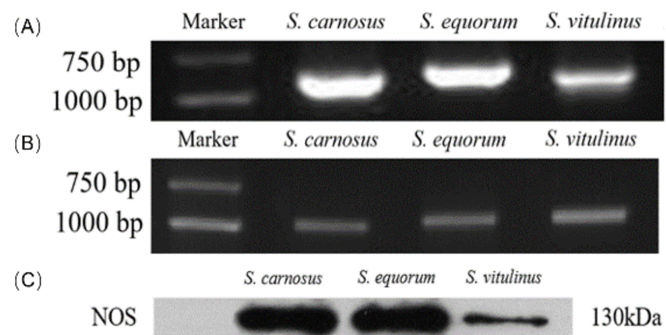

Fig. 1.
(A) The PCR products of DNA electrophoresed on a 2.0% agarose gel.
(B) The PCR products of cDNA electrophoresed on a 2.0 % agarose gel.
(C) Western blot analysis of the crude enzyme extracted from three CNS species.

Notes