

PRELIMINARY STUDY ON THE MECHANISM OF GLOBAL REGULATORS MEDIATED NITRITE REDUCTION IN *LACTOBACILLUS PLANTARUM* FQR

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I. INTRODUCTION

The food additive nitrite is commonly used in meat curing as a food preservation method. However, the excessive nitrite residual causes the problems of food safety. Although some additives can substitute for nitrite in a certain extent, but there is no a substance that can completely substitute the nitrite in meat products. Hence, reducing the nitrite residual was very advantageous to enhance safety of fermented meat.

L. plantarum is closely related to human life, and also commonly used in fermented food. It has been reported that *L. plantarum* has the ability to degrade nitrite^[1]. However, the nitrite metabolism mechanism of *L. plantarum* is unclear. Bacteria generally sense and respond to changes in many different environmental conditions by TCSs. A typical TCS consists of 2 proteins: a histidine protein kinase (HK) (sensor kinase) and a response regulator (RR). Earlier studies have found that there were at least 13 TCSs in *L. plantarum* WCFS1, and that these TCSs were associated with bacteriocin production and quorum sensing^[2]. However, It is not clear whether the TCS is involved in nitrite metabolism of *L. plantarum*.

II. MATERIALS AND METHODS

L. plantarum FQR was selected as the subject of this study; it was originally laboratory-isolated from Chinese fermented meat (GenBank:KX033802.1).

Cells at the stationary phase (16 h) were grown in MRS broth with or without nitrite. Samples were harvested by centrifugation. The cell suspensions were added to liquid nitrogen by grinding. Then, the total RNA was isolated using an RNA mini kit (TaKaRa, Dalian, China). We detected expression of target genes at the mRNA level in different nitrite concentrations by using real time PCR. Relative transcript levels were calculated using the $2^{-\Delta\Delta Ct}$ method.

III. RESULTS AND DISCUSSION

The NarX-NarL and NarQ-NarP sensor-response regulator pairs control *Escherichia coli* (E. Coli) gene expression in response to nitrate and nitrite^[3]. Our group compared the amino acid sequence of two pairs of *L. plantarum* TCSs (HK4-RR4, HK6-RR6) with E. Coli (NarX-NarL, NarQ-NarP) by the NCBI blastp.

The results showed that:

1. The basic characteristics of lactic acid bacteria with nitrite lowering function

The results showed that, In the verification experiment of TCS factors, it was found that FQR contained 13 TCSs and selected HK4, RR4, HK6, RR6, HK5 and RR5 that might have the effect of nitrite reduction to carry out the follow-up experiments.

2. Correlation analysis of NiR and global regulation factors under acid stress, bile salt stress, NaNO₂ stress and NaCl stress

HK4-RR4, HK6-RR6 TCSs and luxs in *L. plantarum* showed the regulation system of nitrite metabolism. The fundamental role of TCSs in *L. plantarum* suggests a new research direction for its metabolic regulation in different natural environments.

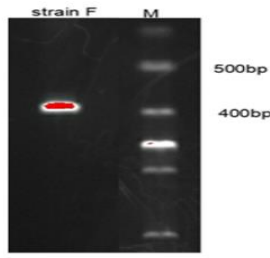


Fig.1 *LuxCS* DNA-electrophoretic diagram

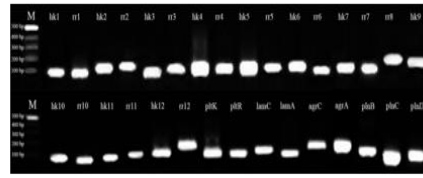


Fig.2 Verification results of TCSs

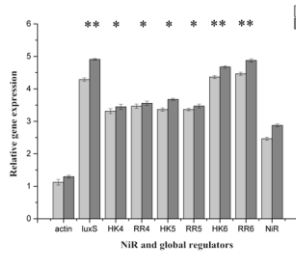


Fig.3 Gene expression of NiR and the global regulatory factors under acid stress

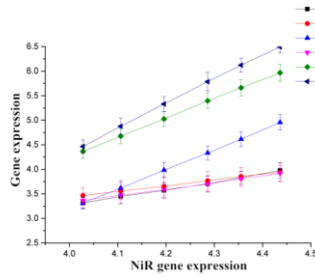


Fig.4 Correlation between global regulators under acid stress

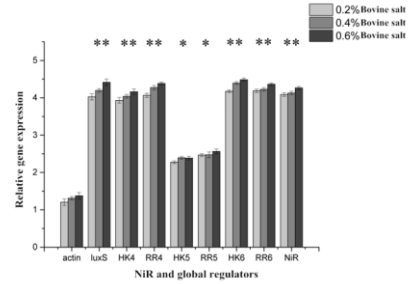


Fig.5 Gene expression of NiR and the global regulatory factors under bile salt stress

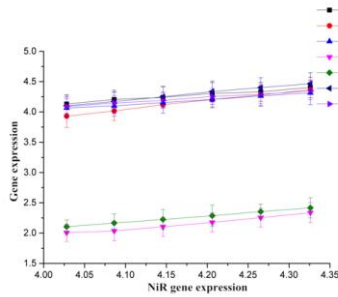


Fig.6 Correlation between NiR and the global regulatory factors under bile salt stress

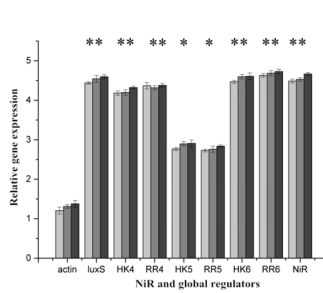


Fig.7 Gene expression of NiR and the global regulatory factors under NaNO₂ stress

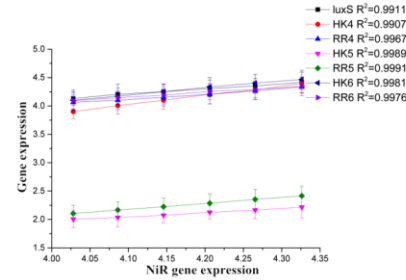


Fig.8 Correlation between NiR and the global regulatory factors under NaNO₂ stress

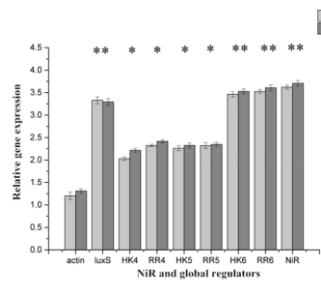


Fig.9 Gene expression of NiR and the global regulatory factors under NaCl stress

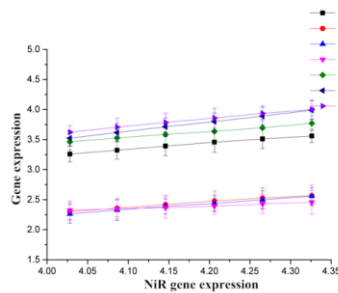


Fig.10 Correlation between NiR and the global regulatory factors under NaCl stress

IV. CONCLUSION

The expression levels of these genes (hk4, rr4, hk6 and rr6) in *L. plantarum* significantly increased with the increase of nitrite concentration. Hence, the HK4-RR4 and HK6-RR6 of *L. plantarum* TCSs may be involved in the regulation of nitrite metabolism.

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