

A NEW DISCOVERY OF NITRITE METABOLISM IN LACTOBACILLUS PLANTARUM

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Abstract – Nitrite is a commonly used additive in fermented meat products, but the excessive nitrite residues in food can cause food safety problems. *Lactobacillus plantarum* (*L. plantarum*) is often found in fermented food, and has the function of degrading nitrite, but its metabolic mechanism is still unclear. Two-component signal system (TCS) plays a role in regulation of many physiological metabolic processes. In this paper, the TCSs of *L. plantarum* FQR to nitrite were investigated. The results showed that the expression levels of these genes (hk4, rr4, hk6 and rr6) in FQR significantly increased with the increase of nitrite concentration. HK4-RR4 and HK6-RR6 TCSs in *L. plantarum* may be involved in the regulation system of nitrite metabolism. It will be a new discovery to verify the nitrite metabolism in *L. plantarum* so as to reduce the food safety risks.

Key Words – Two-component signal system, starter culture, fermented meat

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 $2^{-\Delta\Delta C_t}$ 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 28% of identity between NarX and HK4 (YP_004889330), 33% of identity between NarL and RR4 (YP_004889329); 23% of

identity between NarQ and HK6 (YP_004889716), 34% of identity between NarP and RR6 (YP_004889715). If the amino acid sequence identity of two proteins exceeds 30%, it can be regarded as a homologous protein. Moreover, it was found that RR4 and RR6 were both DNA binding response regulators, belonging to the family of NarL/FixJ proteins by the conserved domain analysis. Therefore, we speculated that HK4-RR6 and HK6-RR6 in *L. plantarum* may have the similar functions as NarX-NarL and NarQ-NarP in *E. coli*, involved in nitrogen metabolism regulation, but the exact functions of TCSs still need further study.

A similar trend in the relative gene expression indicated that the expression levels of these genes (hk4, rr4, hk6 and rr6) in FQR significantly increased with the increase of nitrite concentration compared with control group ($p<0.05$) (Fig. 1). However, the gene expression values of hk4 and rr4 showed no significant difference between 0.045% and 0.075% nitrite ($p>0.05$), and appeared a further increase with greater nitrite concentration. It may be that the strain has a buffering process to respond to external stimuli. It is noteworthy that the gene expression values of k6 and rr6 showed significant difference in the different nitrite concentrations ($p<0.05$). Moreover, the increase of nitrite concentration was accompanied by the increase of gene expression. Additionally, the gene expression of hk4 and hk6 in the same nitrite condition exhibited significant ($p<0.05$) decreases compared with that of rr4 and rr6, respectively. This difference may be due to the delay of phosphorylation signal transmission.

In summary, both HK4-RR4 and HK6-RR6 TCSs 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.

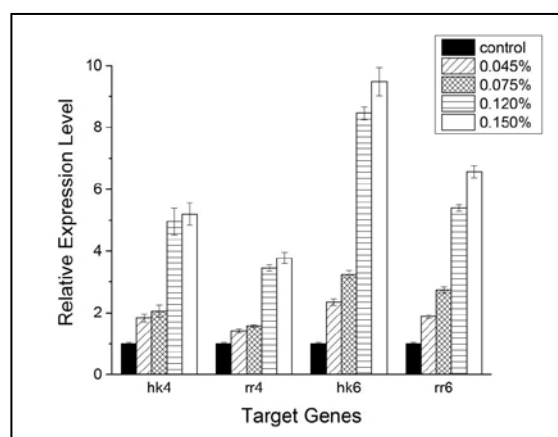


Figure 1. Expression profile of the genes (hk4, rr4, hk6 and rr6) under different nitrite conditions.

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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