THE ROLE OF PHOP/PHOQ SYSTEM IN REGULATING ACID TOLERANCE RESPONSE IN ESCHERICHIA COLI O157:H7

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I. INTRODUCTION

Escherichia coli O157:H7 (*E. coli* O157:H7) is a major foodborne and zoonotic bacterial pathogen. Beef cattle are the main source of *E. coli*, and consumption of contaminated beef may cause foodborne illness outbreaks. Thus, the use of organic acids such as lactic acid, has been long employed in the meat industry as decontamination treatment. However, the unintentional contact of acids with water or fluids drained from carcasses in meat plants may result in the formation of acidic tolerance induction conditions. In addition, the decline of pH (as low as 5.4) is a natural acidification process during the conversion of muscle into meat. *E. coli* cells exposed to moderate acidity acquire therefore increased resistance to challenges comprising extreme acidic conditions [1]. Besides, low temperature and high organic content are also important factors influencing the development of acid tolerance response (ATR) in *E. coli* O157:H7. Two-component regulation system is an important way for bacteria to sense signals from external environment and transmit them to the bacterium, thereby stimulating a series of regulatory mechanisms. So, this study aimed to address two issues relevant to beef safety. Firstly, the acid tolerance of *E. coli* O157:H7 in mild acid, high protein content and low temperature storage media was investigated. Secondly, the role of PhoP/PhoQ in the regulation of amino acid metabolism was investigated, which may contribute to enhanced acid resistance.

II. MATERIALS AND METHODS

Meat extract (ME) medium was prepared firstly [2]. Then, WT and *phoP* mutant strains (Δ*phoP*) originated from different treatments (non-adapted grown in LB; acid-adapted grown in LB; non-adapted grown in ME; and acid-adapted grown in ME) were harvested. *D*-values (min) of acid resistance and quantification of gene expression were also determined [3]. Data were analyzed using the MIXED procedure (SAS, Version 9.2) and plotted by SigmaPlot 10.0 software.

III. RESULTS AND DISCUSSION

D-value of *E. coli* O157:H7 grown in LB medium at pH 7 was 62 min at 37 ℃ (Fig. 1A). After pre-adaptation in acidic conditions at pH 5.4 in ME medium, *D*-value increased from 80.14 min to 205.12, resulting in ATR. Nutrients and temperature of meat during slaughter and processing also affected acid resistance of *E. coli* O157:H7. The occurrence of ATR was higher in cells grown in ME than in LB medium. This may be due to the pathway activated by some nutrients in ME medium promotes ATR. In addition, ATR was limited by low temperature (Fig. 1B). This result proved the necessity of maintaining low temperature during slaughtering as well as meat processing and supply chain. The absence of PhoP/PhoQ system reduced the emergence of ATR, while no significant change was observed in the control group, suggesting that acid adaptation and PhoP/PhoQ system synergistically improved the acid tolerance ability of *E. coli*. Moreover, it indicated that the PhoP/PhoQ TCS can sense H^+ under acidic conditions and activate the acid resistance. However, acid-tolerance of *E. coli* was not completely lost in Δ*phoP*, suggesting that the PhoP/PhoQ TCS might not be the only acid signal transduction system in *E. coli*. Besides PhoP/PhoQ TCS, other acid-resistant mechanisms may play

a protective role in the survival of *E. coli*. Amino acid metabolism played a role in physiological process for maintaining intracellular pH homeostasis. The expression of *adiA* encoding arginine decarboxylase and *cadA* encoding lysine decarboxylase in acid-preadapted cells was upregulated by 21 and 5.7 times than in non-preadapted cells, respectively (Fig. 1C). After deletion of *phoP*, the expression of *adiA* and *cadA* decreased. The *gadA* gene, which encodes glutamic acid decarboxylase, did not show consistent results. These results showed that the PhoP/PhoQ system was activated under acidic conditions and regulated arginine and lysine metabolism to enhance the acid tolerance of *E. coli*.

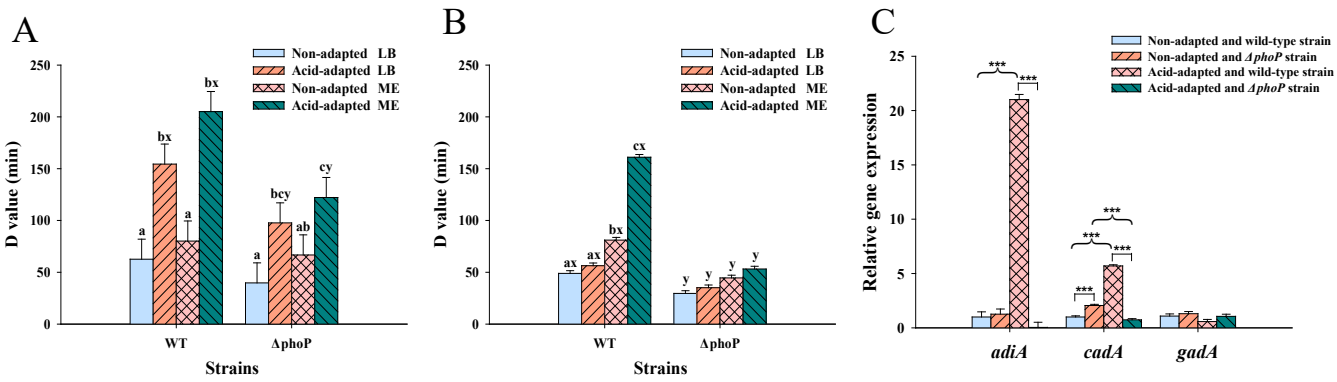

Figure 1. *D*-values (min) for acid-treated (pH = 2, LB medium) cells and the relative expression levels of *adiA*, *cadA* and *gadA* genes (A: 37 ℃; B: 10 ℃; C: 37 ℃).

[a-c] Means different induce treatments (non-adapted & LB, acid-adapted & LB, non-adapted & ME, acid-adapted & ME) differ at $P < 0.05$. [x-y] Means WT and Δ*phoP* differ at $P < 0.05$. An *** means $P < 0.001$. The bar of brackets means significant difference in gene expression between WT and Δ*phoP*. The bar of braces means difference between non-adapted and acid-adapted strains. Relative gene expression of non-adapted *E. coli* O157:H7 was set at 1.0.

IV. CONCLUSION

The medium of meat extract provided a better simulation of the slaughter environment and showed a stronger capacity for acid induction. PhoP/PhoQ system could sense the changes of H^+ and improved the ATR of *E. coli* through arginine and lysine metabolism while ATR could be inhibited by low temperature. In addition to PhoP/PhoQ system, there are other regulatory mechanisms involved in the ATR of *E. coli*.

ACKNOWLEDGEMENTS

This work was supported by a special intergovernmental project for international cooperation in Science, Technology and Innovation (2019YFE0103800), and earmarked fund for China Agriculture Research System (CARS-37).

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