ACID TOLERANCE RESPONSE OF *LISTERIA MONOCYTOGENES* IN DIFFERENT PHS AND CONCENTRATIONS OF LACTIC ACID

Yunge Liu, Pengcheng Dong, Yimin Zhang* and Xin Luo*

College of Food Science and Engineering, Shandong Agricultural University, Taian, Shandong, 271018, PR China;

*Corresponding authors email: ymzhang@sdau.edu.cn; luoxin@sdau.edu.cn.

I. INTRODUCTION

Listeria monocytogenes is a gram positive, food-borne pathogen. It can cause the diseases in both human and animals, which has been associated with a number of fatal outbreaks in recent years. Organic acids are widely used in meat industry to control pathogens [1]. Generally, lactic acids are employed as washes to decontaminate meat surfaces. *L. monocytogenes* growing in the mild acid environment could increase the resistance to the lethal pH condition in the stomach, and this phenomenon could be called acid tolerance response (ATR) [2]. This reaction will lead to the occurrence of high-risk pathogenic bacteria, which is harmful to the population health. In our previous study, it showed 4.75 mM lactic acid at pH 6.0 resulted in a high ATR in 1/2 a and 4b strains of *L*. *monocytogenes*, however, which pH and concentration of lactic acid can cause the highest ATR has not been reported yet [3]. Therefore, the objective of this study is to further explore the ATR in *L. monocytogenes* as a result of the exposure of lactic acid, and to find out the optimal condition which can trigger the highest ATR among three pHs (5.5, 6.0. 6.5) and three concentrations (3mM, 4.75mM and 15mM) of lactic acid.

II. MATERIALS AND METHODS

The bacterial strain (*L*. *monocytogenes* serotype 4b) was kindly provided by Animal Products Processing Laboratory, NAU. The growth conditions were set as two controls and 3 acid treatments as followed. The baseline control treatment was 100 ml of BHI (pH 7.4) without dextrose. HCl control treatments were 100 ml BHI without dextrose adjusted to pH 5.5, 6.0 and 6.5 with HCl. Lactic acid treatments were 100 ml BHI without dextrose added 3 mM, 4.75 mM and 15 mM of L-lactic acid and which were adjusted with HCl to pH 5.5, 6.0 and 6.5, respectively. Growth curves of bacteria in above media were conducted by OD measurements (600 nm) and viable count data hourly. The cells of ATR measurements were harvested at 8h (mid-log phase) by centrifugation (5000×g for 10 min; 5804R, Eppendorf, Germany) at 4 °C. And the bacteria were transferred to the broth at pH 3.0 (BHI without dextrose) and challenged for one hour, and then percentage survival of the bacteria was recorded as described by the previous study [3]. Statistical analysis was carried out with the PASW Statistics 18. The data were analyzed by two-way ANOVA. Differences were considered significant at $P < 0.05$.

III. RESULTS AND DISCUSSION

Growth curves of *Listeria monocytogenes* serotype 4b in various treatments are shown in Fig. 1. All the curves showed S-shape basically and the mid-log phase achieved at about 8h. The optimal pH for growth of *L. monocytogenes* was in the pH 7.4 (neutral range) and as the pH of the medium declined, the growth of cells slowed down (Fig. 1). Lactic acids exerted a more deleterious effect on *L. monocytogenes* than HCl, and as the concentration of lactic acid increases, the growth of cells slowed down. This is because mild acids could lead to a lower intracytoplasmic pH and a higher intracytoplasmic acid anion concentration [4].

As is shown in Fig. 2, 4b of *L. monocytogenes* exhibited the acid resistance (pH 3; P <0.05) in both HCl control and organic acid treatments. Habituation at pH 5.5 resulted in the highest survival of the cells, and the survival decreased as the acid concentration increased, so that 15mM lactic acid induced the strongest ATR at this pH of 5.5. In consistent with our study, it has been reported that within a range of pH 4.0 to 6.0, pH 5.5 induced the strongest acid tolerance response of *L. monocytogenes* [5], however, it was not an effect derived from organic acids. When pH conditions were 6.0 and 6.5, the highest ATR was found when cells were exposed to 3mM lactic acid, meanwhile, no significant ($P > 0.05$) acid tolerance was induced after exposure to 4.75 mM and 15 mM of lactic acid. These results indicate that the effect of lactic acids on acid resistance of *L. monocytogenes* is largely related to the pH conditions.

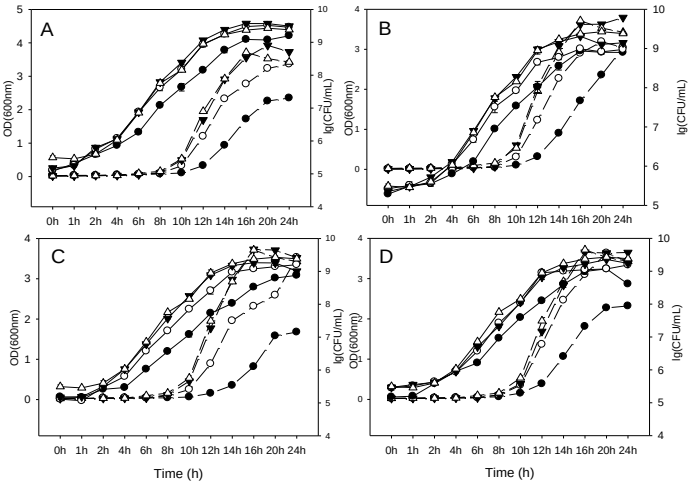

Fig. 1 Comparison of growth curves for *Listeria. monocytogenes* serotype 4b in various acid concentration (37°C). Dotted lines are OD_{600} while solid lines indicate plate count numbers. Symbols: ●, BHI at pH 5.5; ○, BHI at pH 6; ▼, BHI at pH 6.5; △, BHI at pH 7.4. Panel A: 3 mM lactic acid; B: 4.75 mM lactic acid; C: 15 mM lactic acid; D: HCl without lactic acid.

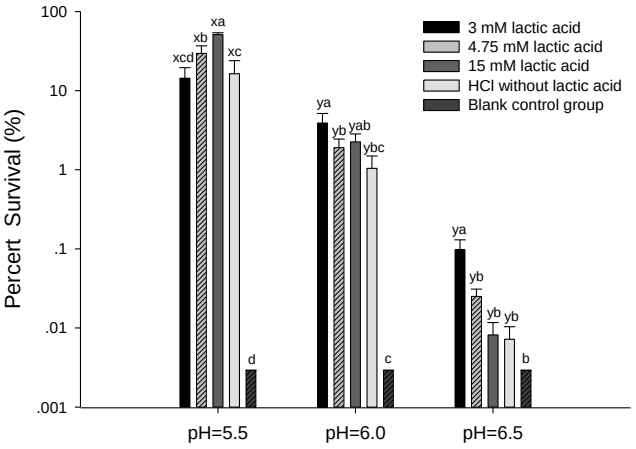

Fig. 2 Percent survival of habituated log-phase *Listeria monocytogenes* serotype 4b after 1 h challenge at pH 3.0. ^{xyz} Means within the same concentration of lactic acid with a different letter differ ($P < 0.05$); ^{abc} Means within the same pH with a different letter differ ($P < 0.05$).

IV. CONCLUSION

In this study, the growth of *L. monocytogenes* serotype 4b slowed down, as expected, with the decrease of pH and the increase of lactic acid concentration in the growth media. Compared with pH 6.0 and 6.5, pH 5.5 at a lactic acid concentration of 15mM could induced the strongest acid tolerance response of L. *monocytogenes*, and the effect derived from lactic acid significantly higher than HCl ($P < 0.05$). Therefore, the processing environment of the factory should take into account the acid tolerance phenomenon of *L. monocytogenes* at optimal acid concentrations and pH levels. In future study, mechanism exploration via determination of the intracellular pH and lactic acid anions levels in the habituated cells, can make a better understanding of the acid tolerance response in *L. monocytogenes*, and can finally promote a more sensible estimation of food safety risk.

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