Behavior of *Burkholderia thailandensis* (*Burkholderia pseudomallei* surrogate) in Acidified Conditions by Organic Acids Used in Ready-to-Eat Meat Formulations under Different Water Activities

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Abstract

This study evaluated the antimicrobial effects of meat processing-related organic acids on *Burkholderia thailandensis* (*Burkholderia pseudomallei* surrogate) with different water activities. *B. thailandensis* KACC12027 (4 log CFU/mL) was inoculated in microwell plates containing tryptic soy broth pH-adjusted to 4, 5, 6, and 7 with ascorbic acid, citric acid, and lactic acid and with water activities adjusted to 0.94, 0.96, 0.98, and 1.0 with NaCl, followed by incubation at 35°C for 30 h. The optical density (OD) of the samples was measured at 0, 3, 6, 12, 24, and 30 h at 595 nm to estimate the growth of *B. thailandensis*. Growth of *B. thailandensis* was observed only at water activity of 1.0. In general, more bacterial growth (p<0.05) was observed at pH 6 than at pH 7, and the antimicrobial effects of the organic acids on *B. thailandensis* were in the following order: Ascorbic acid > lactic acid > citric acid after incubation at 35°C for 30 h. These results indicate that organic acids in meat processing-related formulations should be useful in decreasing the risk related to an emerging high risk agent (*B. pseudomallei*).

Key words: *Burkholderia thailandensis*, *Burkholderia pseudomallei*, dissociation constant

Introduction

*Burkholderia pseudomallei* is a Gram-negative bacterium which is usually isolated from soil, and the pathogen has been known to be related to melioidosis with significant morbidity and mortality up to 90% (Eoin West et al., 2008). This bacterium has recently brought attentions in food supply environments because *B. pseudomallei* shows broad host range and has caused disease in meat producing animals such as cattle, goats, and pigs (Sprague and Neubauer, 2004). In addition, animal-to-human transmission has also been reported, and most cases resulted in fatalities (Choy et al., 2000; Idris et al., 1998). Thus, the food contamination with *B. pseudomallei* is now considered as unavoidable (Dance, 2000). Li et al. (1994) also suggested that the meat from animals infected with *B. pseudomallei* would endanger the public health, and Zinchenko et al. (2008) recently conducted a study of *B. pseudomallei* on meat. In fact, there were melioidosis outbreaks caused by the ingestion of contaminated particles (Bassetti et al., 2005), drinking water (Inglis et al., 1998) and human breast milk (Ralph et al., 2004), and the recent risk assessment by Fosse et al. (2008) characterized *B. pseudomallei* as a bacterial hazard in European pork slaughter houses.

The number of infection cases with *B. pseudomallei* increase after flooding, and monsoon rainfalls are believed to be responsible for spreading *B. pseudomallei*, which leads to a more severe infection in humans (Currie and Jacups, 2003; Ketterer et al., 1975). Taken together, it may suggest the possible ingestion of *B. pseudomallei* through animal origin foods such as pork and beef, especially for the region that has monsoon rainfalls. Therefore, the fate of *B. pseudomallei* needs to be studied under meat processing-related conditions. However, *B. pseudomallei* can be studied only in Biosafety Level (BSL)-3 facilities in many countries due to its high mortality rate (Bossi et al., 2004). *Burkholderia thailandensis* is genetically and morphologically very similar to *B. pseudomallei*, but it is not pathogenic to human (Qazi et al., 2008; Wuthiekanun et al., 1996), and *B. thailandensis* has also similar environment distribution with *B. pseudomallei* (Wuthiekanun et al., 1996; Yu et al., 2006). The...
differences between two species are only variations of arabinose- assimilation operon and the production of capsular polysaccharide (Haraga et al., 2008; Reckseidler et al., 2001). Hence, B. thailandensis has been used in recent studies related to physiological activities and pathogenic characteristics as a surrogate bacterium of B. pseudomallei (Haraga et al., 2008; Qazi et al., 2008; Thongdee et al., 2008; West et al., 2008; Yoon et al., 2010).

A variety of antimicrobials have been used to improve the food safety of manufactured foods (Feng et al., 2010). Most ready-to-eat meat products may contain various antimicrobials such as sodium chloride, lactic acid, nitrite and lactate/diacetate salts to inhibit Listeria monocytogenes growth (Glass et al., 1989; Mbandi and Shelef, 2002). In addition, the decrease of product pHs with organic acids such as ascorbic acid, citric acid, lactic acid, and acetic acid has been suggested in many studies to control pathogens. The previous studies by Calicioglu et al. (2002) and Yoon et al. (2005) showed that the treatments containing ascorbic acid, followed by heating at 60°C increased destruction of Escherichia coli O157:H7 in beef jerky slices. Recent studies also showed that the obvious antimicrobial effects of ascorbic acid and citric acid with chemical tenderizers on E. coli O157:H7 cells during marination at 4°C and cooking (Mukherjee et al., 2008, 2009; Yoon et al., 2009).

This study evaluated the antimicrobial activity of meat processing-related organic acids on emerging food-related agent (B. pseudomallei) using a surrogate (B. thailandensis) under different water activities.

**Materials and Methods**

**Preparation of inoculum**

B. thailandensis KACC 12027 (Korean Agricultural Culture Collection, Korea) stored as a frozen culture at -70°C was cultured in 10 mL of tryptic soy broth (TSB; Difco, Becton Dickinson and Company, USA) at 35°C for 24 h. The 0.1 mL of the culture was then transferred into 10 mL of TSB followed by subculture at 35°C for 24 h. Stationary phase cells were serially diluted with a saline solution to obtain an inoculum size of 4 log CFU/mL.

**Treatments**

To prepare various organic solutions with different levels of pH and water activities, the pH of TSB was adjusted to 4, 5, 6, and 7 with ascorbic acid (Sigma-Aldrich Corp., USA), citric acid (Duksan Pure Chemical Co., Ltd., Korea) and lactic acid (Junsei Chemical Co., Ltd., Japan), respectively, and the water activity for each pH level of the solutions was adjusted to 0.94, 0.96, 0.98, and 1.0 with NaCl (Duksan Pure Chemical). This resulted in a total of 16 combinations in a complete factorial design for each organic acid solution.

**Inoculation and measurement of optical density**

Each combination of organic acid solution was placed in wells of 96 micro-plate, and B. thailandensis was inoculated in each well to obtain 2 log CFU/mL. The micro-plates were then incubated at 35°C for 30 h. The OD (optical density) values of the samples were repeatedly measured at 0, 3, 6, 12, 24, and 30 h at 595 nm with a micro-plate reader (Bio-Tek®, ELx 808, Korea).

**Statistical analysis**

This experiment was repeated twice with two samples in each repeat. The OD values in interactions among the fixed factors such as organic acid solutions, pH, water activity and incubation time were analyzed by the mixed model procedure of SAS® version 9.2 (SAS Institute, Cary, USA). The type III F-test was used to remove random effects in the model at alpha=0.05 by the forward stepwise method, and the interaction effects of fixed effects and random effects were also examined in the type III F-test. Multiple mean comparisons among the interaction (organic acid pH×water activity×incubation time) were performed with the pairwise t-test at alpha=0.05.

**Results and Discussion**

Bacterial growth of B. thailandensis was observed only 1.0 of water activity of solutions (F-value=864.0; p<0.0001) (Figs. 1-3). Thus, data are presented and discussed only for organic acids which had 1.0 of water activity in the text. Although bacterial growth was observed in some samples treated with organic acids, the growth appeared after 12 h of lag phase duration, regardless of the type of organic acid (Figs. 1-3).

In the conditions treated with ascorbic acid, only the samples adjusted to pH 6 and 7 with the organic acid showed dramatic bacterial growth (p<0.05) of B. thailandensis after lag phase duration, but not below pH 5 (Fig. 1B). However, OD995 values of B. thailandensis was significantly higher (p<0.05) in pH 6 of TSB broth (0.625±0.078) than in TSB (0.391±0.102) with pH 7 after incubation at 35°C for 30 h (Fig. 1B). The similar result was also observed under the conditions of TSB treated with lactic acid, and higher (p<0.05) OD995 value (0.156±0.022) was observed