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The Effect of Acid Adaptation Conditions on Acid Tolerance Response of *Escherichia coli* O157: H7

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Abstract: This study examined the effects of acid shock and acid adaptation on acid tolerance of *E. coli* O157: H7 932 strain. *E. coli* O157: H7 were exposed to acid at pH 4.5, 5.0 or 5.5 for acid adaptation or acid shock in tryptic soy broth (acidified with 6 N HCl). Acid shocked cells were incubated for 1, 2, 3 or 4 h while acid adapted cells were incubated for 18 h at 37°C and then acid tolerance of acid shocked and acid adapted cells were determined by exposing to acid challenge of pH 2.5 or 3.0 in tryptic soy broth. Acid shocked, acid adapted and control cells were incubated at 37°C and survival of the cells at 0, 2, 4, 6, 7, 24 and 48 h was determined.

Acid shock increased acid tolerance of *E. coli* O157: H7 at pH 3.0. However, acid tolerance was not induced in acid adapted cells at either pH 2.5 or 3.0. Acid shocked cells exposed to acid at pH 4.5 for 2 h had the highest acid tolerance ($P < 0.05$).

The results indicated that exposure to acidic environments (pH 4.5- 5.5) for a short time increased acid tolerance of *E. coli* O157: H7. Acid shocked cells of *E. coli* O157: H7 may be considered in fermented foods, fruit juices and for acid spray process in the meat industry.

Key Words: *E. coli* O157: H7, Acid shock, Acid adaptation, Adaptation time

Aside Adaptasyon Koşullarının *Escherichia coli* O157: H7'nin Aside Tolerans Kazanması Üzerindeki Etkisi

Özet: Bu çalışmada, asit şoku veya aside adaptasyon işleminin *E. coli* O157: H7 932 suşunun aside tolerans kazanması üzerindeki etkileri incelenmiştir. Duraklama fazı hücreleri pH'sı 6 N HCl ile 4.5, 5.0 ve 5.5' e ayarlanmış tryptic soy broth besiyeri içinde tutulmuştur. Daha sonra asit şokuna maruz bırakılan hücreler 1, 2, 3 ve 4 saat, aside adapte edilen hücreler ise 18 saat 37°C'de inkübe edilmiştir. Asit şoku ve aside adapte edilen hücrelerin aside toleransı, pH 2.5 ve 3.0'te tryptic soy broth besiyeri içinde tespit edilmiştir. Asit şokuna maruz bırakılan kültür, aside adapte edilen kültür ve kontrol kültürü daha sonra 37°C'de inkübe edilmiş ve 0, 2, 4, 6, 7, 24 ve 48 saat sonra canlı sayımları yapılmıştır.

Asit şoku *E. coli* O157: H7'nin aside olan toleransını pH 3.0'te artırmıştır. Ancak aside adaptasyon *E. coli* O157: H7'nin aside olan toleransını pH 2.5'te ve pH 3.0'te artırmamıştır. En yüksek aside tolerans, pH 4.5'te iki saat asit şokuna maruz kalan kültürde tespit edilmiştir ($P < 0.05$).

Elde edilen sonuçlar *E. coli* O157: H7'nin asidik ortamlara (pH 4.5- 5.5) kısa bir süre maruz kalması durumunda, aside tolerans kazandığını göstermiştir. Asit şokuna maruz kalmış *E. coli* O157: H7 hücreleri özellikle fermente gıdalar, meyve suları ve et endüstrisinde kullanılan asit spreyi işlemlerinde dikkate alınmalıdır.

Anahtar Sözcükler: *E. coli* O157: H7, Asit şoku, Aside adaptasyon, Adaptasyon zamanı

Introduction

E. coli O157: H7 is an important food-borne pathogen that causes the disease syndromes of hemorrhagic colitis and hemolytic uremic in humans (1). Dairy and beef cattle are major reservoirs of this pathogen. Although undercooked ground beef has been implicated most often in outbreaks of food-borne illness caused by *E. coli* O157:H7, food poisoning outbreaks involving unpasteurized apple juice (2), apple cider (3), mayonnaise (4) and yoghurt (5) have drawn attention to the acid tolerance properties of this bacterium. In addition to the epidemiological data, studies of survival in

acidic foods and broth's indicate that *E. coli* O157: H7 is acid tolerant (6, 7).

Inducible resistance mechanisms of acid tolerance response could increase the resistance of *E. coli* O157: H7 to acidic conditions. The inducible acid survival mechanisms in enterobacteria such as *Salmonella* (8), *Listeria* (9) and *E. coli* (10) have been studied extensively. Briefly acid adaptation or acid tolerance is a phenomenon by which microorganisms show an increased resistance to environmental stress after the exposure to a moderate acid environment (8).

Foster (8) found that exposing log phase *Salmonella* cells to mild (pH 5.8) or moderate (pH 4.5) acid stress for 30 to 60 minutes would protect cells undergoing a subsequent, rapid transition to pH 3.0. There are four levels of acid tolerance. Including log phase cells, acid adapted log phase cells, stationary phase cells and acid adapted stationary phase cells. The acid adaptation of stationary and log phase cells requires protein synthesis. These proteins are encoded by some specific genes and called as acid shocked proteins (11).

Acid tolerance mechanisms of *E. coli* might allow it to survive in acidic foods, throughout the digestive tract and other body sites with acidic environments, such as the urinary tract (12).

In this study influence of adaptation conditions to acid on survival and acid tolerance response of *E. coli* O157:H7 were investigated. Adaptation time and adaptation pH were followed to determine their effects on acid tolerance of *E. coli* O157:H7.

Materials and Methods

Bacterial Strain and Media

E. coli O157:H7 932 strain (a clinical isolate) was provided by M. P. Doyle (Center for Food Safety and Quality Enhancement. Department of Food Science and Technology, The University of Georgia, Griffin, Georgia, USA).

Stock culture was stored at +4°C in tryptic soy agar (TSA, Oxoid) and subcultured every month. Culture was activated from stock culture after two successive transfers of the test organism in tryptic soy broth (TSB, Oxoid) at 37°C for 24 h. After activation, 0.1 ml of inoculum was added to 50 ml of TSB and incubated at 37°C for 18 h. This activated culture was used for experimental studies. Acidified TSB was prepared by adding appropriate amounts of 6 N HCl to give pH values of 2.5, 3.0, 4.5, 5.0 and 5.5.

Acid adaptation, acid shock and control treatment

To determine acid adaptation, *E. coli* O157:H7 was allowed to adapt in TSB adjusted to pH 4.5, 5.0 or 5.5 at 37°C for 18 h. The treatment to induce acid shock response, *E. coli* O157:H7 was incubated in 50 ml TSB (pH 7.0) at 37°C for 18 h and 40 millilitres of the cultures was taken and dispensed equally to four centrifuge tubes. Cultures were centrifuged at 5000 rpm. After the supernatant was discarded, the cell pellets were suspended in 10 ml of pH 4.5, 5.0 and 5.5 TSB for acid shocked cells. The adaptive exposure times was chosen as 1, 2, 3 and 4 h. Samples were incubated at 37°C.

Control cells were cultured in TSB (pH 7.0) for 18 h at 37°C.

Acid challenge study

To examine the effects of acid adaptation or acid shock on the acid tolerance of *E. coli* O157: H7, 0.5 ml of acid adapted, acid shock and control cultures were added to 50 ml TSB acidified to pH 2.5 or 3.0 and incubated at 37°C.

Determination of viability

Viable population of acid adapted, acid shocked and control cultures were enumerated immediately after inoculation to TSB adjusted to pH 2.5 or 3.0 and after different storage times (0, 1, 2, 3, 4, 5 ,6, 7, 24 or 48 h).

Serial decimal dilutions in 0.1 % peptone water were prepared. The viable population of cultures was then determined by plating 0.1 ml of the serially diluted samples on duplicate sorbitol mac conkey agar (SMAC, oxoid) plates. All plates were incubated at 37°C for 24-48 h, and then colourless colonies were enumerated.

Statistical Analysis

Two replications of each experiment were done. Data were subjected to the SPSS 9.0 statistical software (SPSS Inc., Chicago, IL, USA) for analysis of variance and Duncan's multiple range test was used to determine if adaptation pH and time had a significant effect on the population of control, acid adapted, and acid shocked cells.

Results

Acid adaptation: In this study *E. coli* O157: H7 was subjected to acid adaptation at pH 4.5- 5.5 for 18 h at 37°C. Acid adapted and control cells were then exposed to an acid challenge of pH 2.5 or 3.0. Figure 1 shows survival of acid adapted and control cultures of *E. coli* O157: H7 in TSB adjusted to pH 2.5 or 3.0.

Acid adaptation did not enhance acid tolerance of *E. coli* O157:H7 in TSB at pH 2.5 or 3.0 ($P < 0.05$) (Figure 1).

After 7 h exposure to pH 2.5, control cells decreased by 1.4 log units while acid adapted cells at pH 5.5 decreased by 1.5 log units and cells adapted to acid at pH 5.0 or 5.5 decreased by 1.7 log units. All acid adapted and control culture survived the 7 h exposure to TSB at pH 3.0 with little inactivation. These cultures had only a 1.0 log loss of viability. Exposure to pH 3.0 for 24 h decreased the control population of *E. coli* O157:H7 by 2.3 log units while acid adapted cells at pH 5.5 decreased by 2.7 log units, cells adapted to acid at pH 5.0 decreased by 3.1 log units and acid adapted cells at pH 4.5 decreased by 3.5 log units. At pH 2.5 after 24 h at pH 3.0 after 48 h all acid adapted and control cultures were completely inactivated.

Acid shock: Acid-shocked cells were exposed to acid at pH 4.5- 5.5 for 1- 4 h in TSB at 37°C. Acid tolerance of the acid shocked cells was determined in subsequent acid challenge at pH 2.5 or 3.0 in TSB at 37°C. As shown in Figure 2a control cells showed a higher survival rate than acid shocked cells in TSB adjusted to pH 2.5 ($P < 0.05$). Acid shocked cells did not tolerate acid stress in TSB at pH 2.5 compared with control cells and acid shock times did not effect on acid tolerance of *E. coli* O157:H7. After exposure to pH 3.0, acid shocked cells better tolerated acidic stresses over time than control cells (Figure 2b). Among the various acid shock times tested, 2 h of acid shock time at pH 4.5 resulted in the highest increase in the survival of *E. coli* O157:H7 in the subsequent acid challenge at pH 3.0 ($P < 0.05$).

Figure 2b showed that population of acid shocked and control cells of *E. coli* O157:H7 in TSB at pH 3.0 decreased only 0.1- 1 log in 7 h exposure. After 48 h of incubation in TSB at pH 3.0, cells exposed to acid shock at pH 4.5 and acid shock time of 2 h was detectable at a level of 8.0×10^3 cfu/ml while other cultures were completely inactivated. Cells exposed to acid shock at pH 4.5 or 5.0 for 1 h were detectable 9.0×10^1 cfu/ml and 2.0×10^1 cfu/ml respectively while other cultures were completely inactivated after 48 h of incubation. For 3 and 4 h adaptation time after 48 h of incubation in TSB at pH 3.0 all acid shocked and control cultures were completely inactivated and after 24 h of incubation in TSB at pH 3.0, acid shocked cells at pH 4.5 showed greater survival rate than acid shocked cells at pH 5.0, 5.5 and control culture.

These results indicated that the extent of increased acid tolerance was affected by the time of adaptation, adaptation pH and the condition of the subsequent acid challenge. Acid shocked cells better tolerated acidic stress than acid adapted cells and pH 4.5 is a critical pH for inducing acid tolerance of *E. coli* O157:H7.

Discussion

Stationary *E. coli* O157: H7 cells are more resistant to acid than are log-phase cells. The buffering capacity of cytoplasm, low proton permeability and the extrusion of protons from the cytoplasm by a membrane-bound

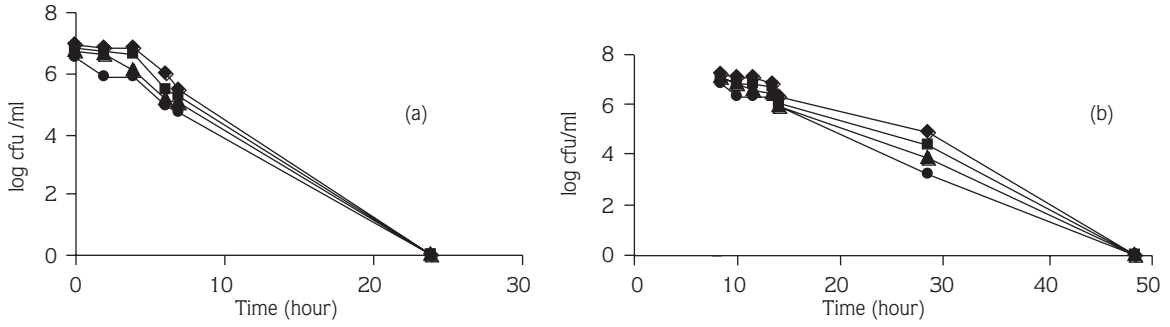

Figure 1. Survival of *E. coli* O157: H7 adapted to acid in TSB for 18 h at pH 5.5 (■), pH 5.0 (▲), pH 4.5 (●) and control culture(◆) inoculated into TSB acidified at pH 2.5 (a) and 3.0 (b) at 37°C.

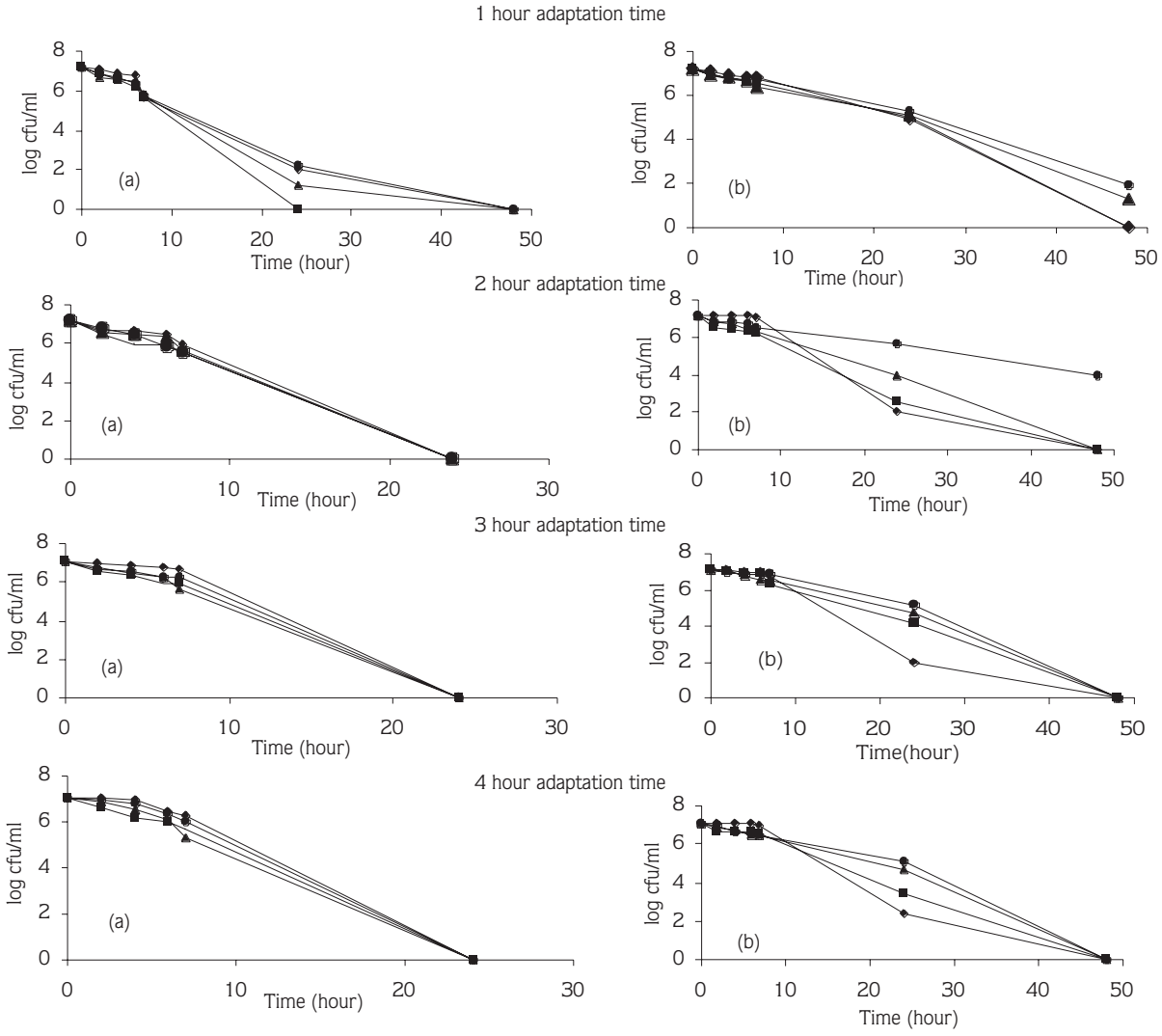

Figure 2. Survival of acid shocked *E. coli* O157: H7 in TSB at pH 5.5 (■), pH 5.0 (▲), pH 4.5 (●) and control culture(◆) for inoculated into TSB acidified at pH 2.5 (a) and 3.0 (b) at 37°C.

proton pump play a very important role in acid resistance mechanism. These systems normally function when protein synthesis inhibitors are present. An alternative mechanism for protection against extreme acid stresses is an inducible pH homeostasis system (acid tolerance response, ATR). The ATR system was found to require protein synthesis and such represents a newly described genetic response to environmental stress (13).

Lin et al. (14) discovered different induced acid resistant system in *E. coli* depending on whether oxidative or fermentative metabolism is occurring. These systems are called as glutamate decarboxylase, arginine decarboxylase, and acid- induced oxidative system.

O'driscoll et al. (15) reported that *L. monocytogenes* exhibited a significant acid tolerance response following a 1 h exposure to mild pH (5.5). Foster (16) reported that *S. typhimurium* had the ability to survive extremely low pH (pH 3- 4) if first adapted to mild pH (pH 5.6- 6.0). These inducible acid resistance mechanisms result in the production of proteins responsible for the prevention or recovery from acid damage to the cell (13).

Results obtained from the present study demonstrate that acid shock increased the acid tolerance of *E. coli* O157: H7 . The extent of increased acid tolerance was affected by the pH and time of adaptation and the conditions of the subsequent acid challenge. Among the various adaptation times and pH tested, generally exposure of *E. coli* O157: H7 to acid at pH 4.5 for 2 h resulted in the maximum increase in acid tolerance which was observed in the subsequent acid challenge at pH 3.0. These conditions could allow for the induction of acid shock proteins in response to acid stress. Heyde and Portalier (17) found that a pH shift from 6.9 to 4.3 induced the production of at least 16 polypeptides. Seven of these were specifically identified as acid shock proteins.

Acid adapted and acid shocked cells did not tolerate acidic stress at pH 2.5 compared with control cells. The acid tolerance response systems protect well at pH 3.0 but have no effect at pH 2.5.

Acid adapted cells in TSB incubated at 37°C for 18 h did not tolerate acidic stresses in TSB at pH 2.5 or 3.0 compared with control cells. These results indicated that acid adaptation did not induce acid tolerance. However Buchanan and Edelson (18) described a procedure for producing acid-adapted *E. coli* O157: H7 cells by growing them in TSB supplemented with 1% glucose. The organism ferment glucose, resulting in acid production and a decrease in pH and cells undergo acid adaptation.

In conclusion acid tolerance of this pathogen is an important point which should be concern to both food processors and clinicians, since *E. coli* O157: H7 may tolerate the acidic environments both in food processes and in human body, and organic acids frequently used to inhibit the growth and survival of pathogens. *E. coli* O157: H7 may survive in various acidic foods as well as the acid sprays or acid dips. The results of this study indicate that acid shocked *E. coli* O157: H7 in foods could survive for a sustained period.

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