# Acid Adapted *Vibrio parahaemolyticus* and *Vibrio vulnificus* Enhance Survival in Acidic Environments

Jaheon Koo
Michael L. Jahncke

**ABSTRACT.** Prior exposure of *Vibrio parahaemolyticus* and *V. vulnificus* cells to pH 5.5 resulted in an adaptive acid tolerance response of these bacteria to pH 4.0. Acid adapted *V. parahaemolyticus* and *V. vulnificus* cells at pH 5.5 for 1 h were reduced by 5 logs within 2 h after exposure to pH 4.0, while non-acid adapted cells were reduced by 4.5 logs within 15 to 30 min after exposure to pH 4.0. Chloramphenicol treated cells were not acid tolerant and were reduced by more than 5 logs within 10 min after exposure to pH 4.0 conditions. This study suggests that development of acid tolerance in *V. parahaemolyticus* and *V. vulnificus* may increase the likelihood of these bacteria to survive the acid environment found in the human stomach. 

**KEYWORDS.** Acid adaptation, *Vibrio parahaemolyticus*, *Vibrio vulnificus*

---

Jaheon Koo is affiliated with University of Arkansas at Pine Bluff, Department of Agriculture, 1200 North University Drive, Mail Slot 4913, Pine Bluff, AR 71601 (E-mail: koo_j@uapb.edu).

Michael L. Jahncke is affiliated with Virginia Seafood Agricultural Research and Extension Center, Virginia Tech, Virginia Sea Grant Program, 102 South King Street, Hampton, VA 23669 (E-mail: mjahncke@vt.edu).

Address correspondence to: Dr. Michael L. Jahncke at the above address.

Journal of Aquatic Food Product Technology, Vol. 15(2) 2006
Available online at http://www.haworthpress.com/web/JAFPT

doi:10.1300/J030v15n02_05

## *INTRODUCTION*

*V. parahaemolyticus* and *V. vulnificus* are naturally occurring bacteria distributed in estuarine environments. They are associated with seafood including molluscan shellfish (DePaola et al., 1994; Motes et el., 1998; DePaola et al., 2000). *V. parahaemolyticus* and *V. vulnificus* infections are associated with consumption of raw or undercooked shellfish and wound infections via seawater (Shapiro et al., 1998; Daniels et al., 2000). Ingestion of *V. vulnificus* from raw oysters can result in 60% mortality in people who are susceptible to this bacterium (Shapiro et al., 1998). Recent *V. parahaemolyticus* outbreaks in the United States were associated with consumption of raw or undercooked oysters harvested from the Washington, Texas, and New York areas (CDC, 1998 and 1999; DePaola et al., 2000). The clinical isolates of *V. parahaemolyticus* collected from Galveston Bay, Texas were serotype O3:K6 (DePaola et al., 2000), commonly associated with *V. parahaemolyticus* illnesses in Asia (Okuda et al., 1997).

Pathogenic bacteria may be subjected to a variety of stresses found in the environment, in foods, and in the human host. The gastric barrier in humans is lethal to most ingested microorganisms (Peterson et al., 1989). Foods high in protein may protect bacteria from the acidic environment thus increasing survival (Waterman and Small, 1998). In addition, reduction of gastric acid through the use of antacids can lower the infective dose of *Vibrio cholerae* needed to cause human illness (Cash et al., 1974). It was also shown to increase the survival of *V. vulnificus* in a gastrointestinal model (Koo et al., 2001).

*V. cholerae*, *V. parahaemolyticus*, and *V. vulnificus* are acid sensitive (Wong et al., 1998; Koo et al., 2000a and 2000b) compared with other enteric pathogens such as *Salmonella typhimurium*, *Salmonella typhi*, *Shigella flexneri*, and *Escherichia coli* O157:H7 (Waterman and Small, 1998). The acid tolerance of enteric pathogens is highest in the late log or stationary phase (Gorden and Small, 1993; Arnold and Kaspar, 1995; Benjamin and Datta, 1995; De Koning-Ward and Robins-Browne, 1995). Acid adaptation enhanced survival of *S. typhimurium*, *Aeromonas hydrophila*, and *E. coli* O157:H7 in acidic foods (Foster and Hall, 1990; Karem et al., 1994; Leyer et al., 1995) and induced cross protection against environmental stresses encountered during food processing, including heat, osmotic, or oxidative stress (Leyer and Johnson, 1993; Ryu and Beuchat, 1998; Garren et al., 1998). Urease, an enzyme responsible for hydrolyzing urea to form carbon dioxide and ammonia,

contributed to higher internal cell pH and enhanced the acid tolerance of *Yersinia enterocolitica* (De Koning-Ward and Robins-Browne, 1995).

Similar characteristics were also found in *V. parahaemolyticus* (Wong et al., 1998). Adaptation of *V. parahaemolyticus* to environment stresses induces cross protection against other environmental stresses (Koga and Takumi, 1995a and b). For example, *V. vulnificus* after exposure to an intermediate cold temperature of 15°C showed higher survival at lower temperatures compared with unadapted cells (Bryan et al., 1999). Protein synthesis played a significant role in protecting the cells against acid stress or cold temperatures (Foster and Hall, 1990; Karem et al., 1994; Wong et al., 1998; Bryan et al., 1999). Protein synthesis induced by acid stresses protects cells from denaturation or damage by maintaining internal pH values close to neutral and preventing or repairing damage to DNA (Karem et al., 1994).

Understanding factors that may affect the survival of *V. parahaemolyticus* and *V. vulnificus* is an important issue for protecting public health. The objectives of the this study were: (1) to evaluate the acid adaptation of *V. parahaemolyticus* and *V. vulnificus* at different growth phases after mild acid exposure to pH 5.5; and (2) to determine if protein synthesis is required for acid adaptation of *V. parahaemolyticus* and *V. vulnificus*.

## *MATERIALS AND METHODS*

### *Bacterial Strains*

Clinical isolates of *V. parahaemolyticus* TX-2103 (serotype O3:K6) and *V. vulnificus* MO-624 were obtained from the US Food and Drug Administration, Dauphin Island, AL. Both strains were maintained at room temperature on $T_1N_1$ agar slants containing 10 g tryptone (Difco Laboratories, Detroit, MI), 10 g NaCl, 20 g bacto agar (Difco), and 1.0 L distilled water with sterile mineral oil overlaid.

### *Acid Tolerance*

Cultures (0.1 ml) were inoculated into a flask containing 100 ml of $T_1N_1$ broth containing 10 g tryptone, 10 g NaCl, and 1.0 L distilled water (pH 7.2) and grown at 35°C with shaking. Mid-log phase (2.5 h), late-log phase (6 h), and stationary phase (18 h) cultures were adjusted to pH 4.5 with sterile 1N HCl, and incubated at 35°C for 1 h with shak-

ing. Aliquots were withdrawn before addition of HCl and after 1 h incubation, and plated onto tryptic soy agar (Difco Laboratories, Detroit, MI) supplemented with 1% NaCl (TSA-1) using a spiral plater (Microbiology International, Frederick, MD) to calculate the percent log survivors. Plates were incubated at 35°C for 18-20 h. The percent log survivors for each growth phase were calculated as follow: (log CFU/ml after 1 h incubation in acidified broth divided by the log CFU/ml before acid challenge) $\times$ 100.

### *Acid Adaptation*

An aliquot of 0.1 ml from overnight cultures was inoculated into a flask containing 100 ml of $T_1N_1$ broth and grown at 35°C with shaking until mid-log phase. The mid-log phase cultures were acidified to mild acid at pH 5.5 using sterile 1N HCl, shaken and incubated at 35°C for 1 h. The cultures were then challenged at pH 4.0 by adding more HCl, and incubating for an additional 2 h. A second culture was challenged to pH 4.0 and incubated at 35°C for 15-30 min with shaking without the intermediate incubation step at pH 5.5. Aliquots were serially diluted in phosphate-buffered saline (PBS), and plated on TSA-1 using a spiral plater. Plates were incubated at 35°C for 18-20 h.

### *Protein Synthesis in Acid Adaptation*

An aliquot of 0.1 ml from the overnight culture was inoculated into a flask containing 100 ml of $T_1N_1$ broth and grown at 35°C with shaking until mid-log phase was achieved. The culture was treated with chloramphenicol (0.5 μg/ml) (Sigma Chemical Co., St. Louis, MO), and then acidified to pH 5.5 with sterile 1N HCl. Cultures were incubated at 35°C for 1 h with shaking. The cultures were challenged to pH 4.0 conditions by adding additional HCl, and incubating for 20 min. A second culture was similarly exposed to pH 5.5 for 1 h followed by pH 4.0, but no chloramphenicol was added. Aliquots were serially diluted in PBS, and plated on TSA-1 using a spiral plater. Plates were incubated at 35°C for 18-20 h.

### *Statistical Analysis*

Comparison of means from four to eight measurements, at a significance level ($p < 0.05$), was performed by a one-way analysis of variance

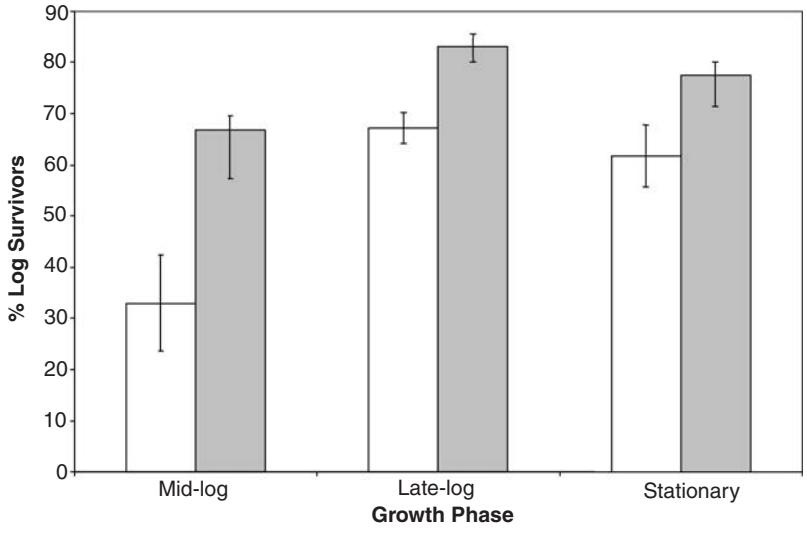
(ANOVA) using SPSS (SPSS Inc., Chicago, IL). Results are expressed as mean values ± standard deviations.

## *RESULTS AND DISCUSSION*

### *Acid Tolerance*

Plate counts of *V. parahaemolyticus* and *V. vulnificus* at pH 7.2 were 7.5 and 6.7 CFU/ml at the mid-log phase, 9.3 and 9.0 CFU/ml at the late-log phase, and 9.5 and 9.6 CFU/ml at the stationary phase, respectively, before challenging them to pH 4.5. Acid tolerance of *V. parahaemolyticus* and *V. vulnificus* was growth phase dependent (Figure 1). *V. parahaemolyticus* was more acid tolerant at each growth phase at pH 4.5 compared with *V. vulnificus*. Acid tolerance of *V. parahaemolyticus* was the greatest ($p < 0.05$) at late-log phase with 83% survivors. There were no differences in acid tolerance of *V. vulnificus* between at late-log phase with 67% survivors and at stationary phase

FIGURE 1. Growth phase-dependent acid tolerance of *V. parahaemolyticus* and *V. vulnificus*. (■) *V. parahaemolyticus* in $T_1N_1$ broth at pH 4.5 and (□) *V. vulnificus* in $T_1N_1$ broth at pH 4.5.



with 62% survivors. Mid-log phase *V. parahaemolyticus* and *V. vulnificus* cultures were the least acid tolerant with 65% and 38% survivors, respectively. In late-log phase, both *V. parahaemolyticus* and *V. vulnificus* were more acid tolerant compared with mid-log phase with 83% and 67% survivors, respectively. At the stationary phase, acid tolerance of *V. parahaemolyticus* and *V. vulnificus* decreased slightly (78% and 62%, respectively).

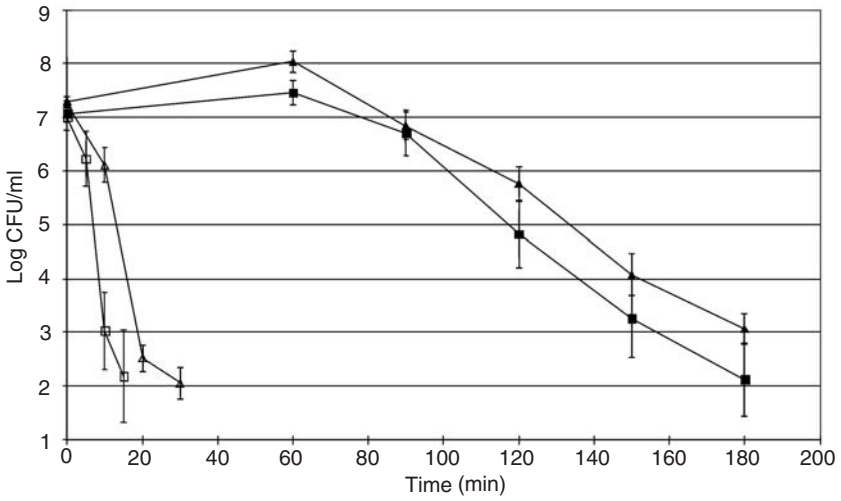
Acid tolerance can be caused by stress factors such as starvation, temperature, salinity, and pH (Arnold and Kaspar, 1995; Benjamin and Datta, 1995). The acid tolerance of *V. parahaemolyticus* and *V. vulnificus* in this study is similar to results reported for multiple strains of *E. coli* O157:H7, *S. flexneri*, and *Salmonella*, that showed increased acid tolerance at the log- or stationary-phases (Arnold and Kaspar, 1995; Benjamin and Datta, 1995; Gorden and Small, 1993). Since the enteric pathogens used in previous studies were exposed for longer periods of time to lower pH values, compared with *V. parahaemolyticus* and *V. vulnificus* used in this study, they had greater survivability and increased acid tolerance compared with *V. parahaemolyticus* and *V. vulnificus*.

### *Acid Adaptation*

Exposure of *V. parahaemolyticus* and *V. vulnificus* to mild acid conditions (pH 5.5) for 1 h enhanced the survival of these bacteria at pH 4.0 (Figure 2) ($p < 0.05$). When the mid-log phase cultures at pH 7.2 were challenged to pH 4.0 without prior exposure to mild acid (pH 5.5), the non-adapted cultures declined 4.5 logs within 30 min. After acid adaptation for 1 h at pH 5.5, and subsequent challenges to pH 4.0 for 2 h, the adapted *V. parahaemolyticus* and *V. vulnificus* cells survived more than 4 times longer at pH 4.0 compared with non-adapted cells ($p < 0.05$). *V. parahaemolyticus* and *V. vulnificus* underwent rapid death when subjected to pH 4.0 without prior adaptation at pH 5.5.

Wong et al. (1998) also showed that exposure of *V. parahaemolyticus* to mild acid conditions (pH 5.0) enhanced survival to subsequent lower acid challenges (pH 4.4). Compared with the non-adapted cells, the acid-adapted *V. parahaemolyticus* showed increased cross-protection against low salinity and thermal inactivation, and decreased the infective dose needed to cause illness in the suckling mouse model (Wong et al., 1998). When *V. vulnificus* was adapted to 15°C prior to challenging it at 6°C, the cells remained viable and culturable, whereas shifting *V. vulnificus* cultures directly from 35°C to 6°C induced a non-culturable state (Bryan et al., 1999). In addition, cold temperature (6°C) adapted

FIGURE 2. Mild acid treatment (pH 5.5) on acid adaptation of *V. parahaemolyticus* and *V. vulnificus* prior to acid challenge (pH 4.0). (▲) acid adapted *V. parahaemolyticus* at pH 5.5; (■) acid adapted *V. vulnificus* at pH 5.5; (△) non-acid adapted *V. parahaemolyticus*; (□) non-acid adapted *V. vulnificus*.




*V. vulnificus* had higher survival rates following freezing at −78°C compared with cultures held at 35°C and then frozen (Bryan et al., 1999).

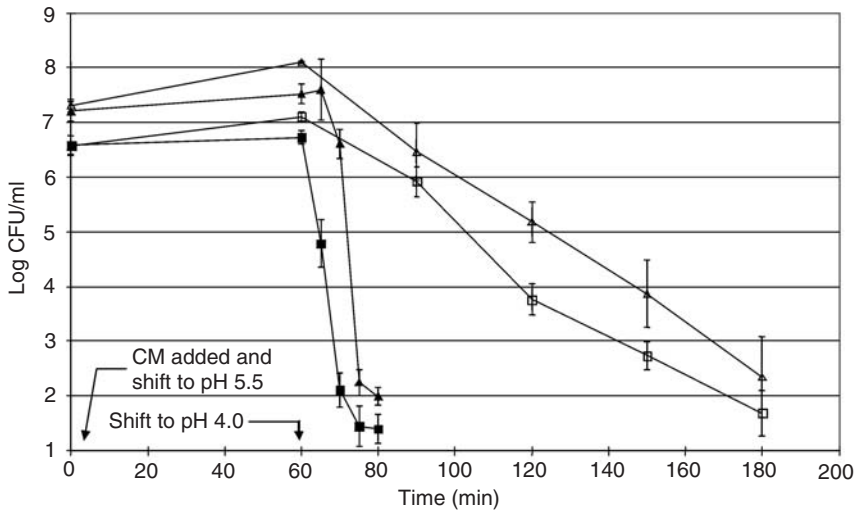
### *Protein Synthesis in Acid Adaptation*

Protein synthesis is required for acid adaptation of *V. parahaemolyticus* (Wong et al., 1998) and cold temperature adaptation of *V. vulnificus* (Bryan et al., 1999). Although this study did not analyze protein profiles during acid adaptation, the addition of the protein synthesis inhibitor, chloramphenicol during the acid adaptation step, prevented acid tolerance adaptation in *V. parahaemolyticus* and *V. vulnificus*. This suggests that protein synthesis in *V. parahaemolyticus* and *V. vulnificus* is required for acid adaptation (Figure 3) ($p < 0.05$). Further investigations are needed to determine the specific proteins involved in the process and how their expression is regulated.

## *CONCLUSIONS*

The adaptive acid tolerance of *V. parahaemolyticus* and *V. vulnificus* may enhance protection from acidic environments or other stresses and

FIGURE 3. Inducible acid tolerance in *V. parahaemolyticus* after treatment with chloramphenicol (CM). (▲) *V. parahaemolyticus* treated with CM; (■) *V. vulnificus* treated with CM; (△) *V. parahaemolyticus* not treated with CM; (□) *V. vulnificus* not treated with CM.




increase survival in such environments. The adaptive nature of *V. parahaemolyticus* and *V. vulnificus* to stress factors could be important because stress-adapted *Vibrio* may have a greater chance to survive the passage through the stomach and increase the risk of *Vibrio* infection. Since stress-adapted *Vibrio* have a tendency to develop cross-protection against other stress factors, post-harvest processing may require steps to minimize exposure of raw oyster to mild stress factors. This study demonstrated that acid adaptation increases the resistance of *V. parahaemolyticus* and *V. vulnificus* to low pH conditions, resulting in potential higher survival in the acid environment of the human stomach.

## REFERENCES

Arnold, K.W. and Kaspar, C.W. 1995. Starvation- and stationary-phase-induced acid tolerance in *Escherichia coli* O157:H7. Appl. Environ. Microbiol. 61: 2037-2039.

Benjamin, M.M. and Datta, A.R. 1995. Acid tolerance of enterohemorrhagic *Escherichia coli*. Appl. Environ. Microbiol. 61: 1669-1672.

Bryan, P.J., Steffan. R.J., DePaola, A., Foster, J.W., and Bej, A.K. 1999. Adaptive response to cold temperature in *Vibrio vulnificus*. Curr. Microbiol. 38: 168-175.

Cash, R.A., Music, S.I., Libonati, J.P., Snyder, M.J., Wenzel, R.P., and Hornick, R.B. 1974. Response of man to infection with *Vibrio cholerae*. I. Clinical, serologic, and bacteriologic responses to a known inoculum. J. Infect. Dis. 129: 45-52.

CDC, Center for Disease Control and Prevention. 1998. Outbreak of *Vibrio parahaemolyticus* infections associated with eating raw oysters-Pacific Northwest, 1997. MMWR 47: 457-462.

CDC, Center for Disease Control and Prevention. 1999. Outbreak of *Vibrio parahaemolyticus* infection associated with eating raw oysters and clams harvested from Long Island Sound-Connecticut, New Jersey, and New York, 1998. MMWR 48: 48-51.

Daniels, N.A., MacKinnon, L., Bishop, R., Altekruse, S., Ray, B., Hammond, R.M., Thompson, S., Wilson, S., Bean, N.H., Griffin, P.M., and Slutsker, L. 2000. *Vibrio parahaemolyticus* infections in the United States, 1973-1998. J. Infect. Dis. 181: 1661-1666.

De Koning-Ward, T.F. and Robins-Browne, R.M. 1995. Contribution of urease to acid tolerance in *Yersinia enterocolitica*. Infect. Immun. 63: 3790-3795.

DePaola, A., Capers, G.M., and Alexander, D. 1994. Densities of *Vibrio vulnificus* in the intestines of fish from the U.S. Gulf Coast. Appl. Environ. Microbiol. 60: 984-988.

DePaola, A., Kaysner, C.A., Bowers, J., and Cook, D.W. 2000. Environmental investigations of *Vibrio parahaemolyticus* in oysters after outbreaks in Washington, Texas, and New York (1997 and 1998). Appl. Environ. Microbiol. 66: 4649-4654.

Foster, J.W. and Hall, H.K. 1990. Adaptive acidification tolerance response of *Salmonella typhimurium*. J. Bacteriol. 172: 771-778.

Garren, D.M., Harrison, M.A., and Russell, S.M. 1998. Acid tolerance and acid shock response of *Escherichia coli* O157:H7 and non-O157:H7 isolates provide cross protection to sodium lactate and sodium chloride. J. Food Prot. 61: 158-161.

Gorden, J. and Small, P.L.C. 1993. Acid resistance in enteric bacteria. Intect. Immun. 61: 364-367.

Karem, K.L., Foster, J.W., and Bej, A.K. 1994. Adaptive acid tolerance response in *Aeromonas hydrophila*. Microbiol. 140: 1731-1736.

Koga, T. and Takumi, K. 1995a. Comparison of cross-protection against some environmental stresses between cadmium-adapted and heat-adapted cells of *Vibrio parahaemolyticus*. J. Gen. Appl. Microbiol. 41: 263-268.

Koga, T. and Takumi, K. 1995b. Nutrient starvation induces cross protection against heat, osmotic, or $H_2O_2$ challenge in *Vibrio parahaemolyticus*. Microbiol. Immunol. 39: 213-215.

Koo, J., DePaola, A., and Marshall, D.L. 2000a. Impact of acid on survival of *Vibrio vulnificus* and *V. vulnificus* phage. J. Food Prot. 63: 1049-1052.

Koo, J., DePaola, A., and Marshall, D.L. 2000b. Effect of simulated gastric fluid and bile on survival of *Vibrio vulnificus* and *V. vulnificus* phage. J. Food Prot. 63: 1665-1669.

Koo, J., Marshall, D.L., and DePaola, A. 2001. Antacid increases survival of *Vibrio vulnificus* and *Vibrio vulnificus* phage in a gastrointestinal model. Appl. Environ. Microbiol. 67: 2895-2902.

Leyer, G.J. and Johnson, E.A. 1993. Acid adaptation induces cross-protection against environmental stresses in *Salmonella typhimurium*. Appl. Environ. Microbiol. 59: 1842-1847.

Leyer, G.J., Wang, L.L., and Johnson, E.A. 1995. Acid adaptation of *Escherichia coli* O157:H7 increases survival in acidic foods. Appl. Environ. Microbiol. 61: 3752-3755.

Motes, M.L., DePaola, A., Cook, D.W., Veazey, J.E., Hunsucker, J.C., Garthright, W. E., Blodgett, R.J., and Chirtel, S.J. 1998. Influence of water temperature and salinity on *Vibrio vulnificus* in Northern Gulf and Atlantic Coast oysters (*Crassostrea virginica*). Appl. Environ. Microbiol. 64: 1459-1465.

Okuda, J., Ishibashi, M., Hayakawa, E., Nishino, T., Takeda, Y., Mukhopadhyay, A.K., Garg, S., Bhattacharya, S.K., Nair, G.B., and Nishibuchi, M. 1997. Emergence of a unique O3:K6 clone of *Vibrio parahaemolyticus* in Calcutta, India, and isolation of Strains from the same clonal group from southeast Asian travelers arriving in Japan. J. Clin. Microbiol. 35: 3150-3155.

Peterson, W.L., Mackowiak, P.A., Barnett, C.C., Marling-Cason, M., and Haley, M.L. 1989. The human gastric bactericidal barrier: Mechanisms of action, relative antibacterial activity, and dietary influences. J. Infect. Dis. 159: 979-983.

Ryu, J.H. and Beuchat, L.R. 1998. Influence of acid tolerance responses on survival, growth, and thermal cross-protection of *Escherichia coli* O157:H7 in acidified media and fruit juices. Int. J. Food Microbiol. 45: 185-193.

Shapiro, R.L., Altekruse, S., Hutwagner, L., Bishop, R., Hammond, R., Wilson, S., Ray, B., Thompson, S., Tauxe, R.V., Griffin, P.M., and the *Vibrio* Working Group. 1998. The role of Gulf Coast oysters harvested in warmer months in *Vibrio vulnificus* infections in the United States, 1988-1996. J. Infect. Dis. 178: 752-759.

Waterman, S.R. and Small, P.L.C. 1998. Acid-sensitive enteric pathogens are protected from killing under extremely acidic conditions of pH 2.5 when they are inoculated onto certain solid food sources. Appl. Environ. Microbiol. 64: 3882-3886.

Wong, H.C. Peng, P.Y., Han, J.M., Chang, C.Y., and Lan, S.L. 1998. Effect of mild acid treatment on the survival, enteropathogenicity, and protein production in *Vibrio parahaemolyticus*. Infect. Immun. 66: 3066-3071.