Effect of pH, NaCl Content, and Temperature on Growth and Survival of Arcobacter spp.

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ABSTRACT

Growth and survival of six human isolates of the pathogenic Arcobacter spp. in the presence of selected environmental factors were studied. Four strains of Arcobacter butzleri and two strains of Arcobacter cryaerophilus were exposed to pH levels of 3.5 to 8.0. Most strains grew between pH 5.5 and 8.0, with optimal growth of most A. butzleri and A. cryaerophilus strains at pH 6.0 to 7.0 and 7.0 to 7.5, respectively. The 24-h optimal growth range in the presence of NaCl was 0.5 to 1.0% for A. cryaerophilus. However, after 96 h, the optimum was between 0.5 and 2.0% NaCl. The optimum range for growth of A. butzleri strains was 0.09 to 0.5% NaCl after 96 h. The upper growth limits were 3.5 and 3.0% NaCl for A. butzleri and A. cryaerophilus, respectively. Survival at 25°C in up to 5% NaCl was noted for A. butzleri 3556 and 3539 and A. cryaerophilus 3256. Decimal reduction times (D-values) at pH 7.3 in phosphate-buffered saline for three A. butzleri strains were 0.07 to 0.12 min at 60°C, 0.38 to 0.76 min at 55°C, and 5.12 to 5.81 min at 50°C. At pH 5.5, decreased thermostolerance was observed, with D-values of 0.03 to 0.11 min at 60°C, 0.30 to 0.42 min at 55°C, and 1.97 to 4.42 min at 50°C. Calculated z-values ranged from 5.20 to 6.28°C. D-values of a three-strain mixture of A. butzleri in raw ground pork were 18.51 min at 50°C and 2.18 min at 55°C. Mild heat (50°C) followed by cold shock (4 or 8°C exposure) had a synergistic lethal effect, reducing more cells than with an individual 50°C treatment or with cold shock temperatures of 12 or 16°C.

Members of the genus Arcobacter, often described as “emerging pathogens,” comprise four species: A. butzleri, A. cryaerophilus, A skirrowii, and A. nitrofugillus (24, 31, 32). These gram-negative, aerotolerant, vibriolike bacteria were first observed by Ellis et al. (15) in veterinary specimens. They are closely related to and were formerly designated as members of Campylobacter. Strains of Arcobacter have been detected in drinking water (21, 26) and muscle foods, including pork, beef, and poultry (3, 4, 11, 14, 22), and in poultry processing plants at levels higher than those of thermophilic Campylobacter spp. (16). They have been clinically associated with abortions and mastitis in livestock (6, 27, 35) in several countries and diarrhea in nonhuman primates (1). A recent study of strains from porcine abortions in Denmark has, in fact, suggested the existence of a new pathogenic Arcobacter species (27).

Arcobacter-associated illness in humans takes the form of persistent diarrhea, gastroenteritis, or occasionally septicemia (19, 35). A. butzleri is the species most often linked to outbreaks of disease in humans. The more significant reports include an outbreak among Italian schoolchildren (33) and acute diarrheal disease in Thai children (30). Severe or long-term diarrhea, sometimes with accompanying bacteremia, was reported among 29 U.S. patients suffering from A. butzleri infection (35). A. butzleri has also been isolated from a newborn with bacteremia (28) and enteric patients with chronic underlying disease (23). Studies have revealed the varied antibiotic response of Arcobacter strains (2, 28), and virulence studies on A. butzleri detected the production of cytotoxins by almost all strains (26).

Foodborne illness affects millions of people in the United States each year. Campylobacter is the leading cause of bacterial foodborne disease according to statistics for the year 2000 released by the Centers for Disease Control and Prevention. The organism was the most frequently detected pathogen in the first 5 years of the survey and was isolated in 36.7% of laboratory-tested cases of diarrhea. Campylobacter infection rates surpassed the incidence of Salmonella- and Shigella-associated disease (9). Although the mortality rate is low in the estimated 2.4 million affected people, 124 to 500 Campylobacter-related deaths occur in the United States each year. In addition, 40% of the cases of Guillain-Barré syndrome are thought to be initiated by Campylobacter infections (7, 8). The closely related Arcobacter spp. are similarly found in foods and are also associated with gastritis. A role for Arcobacter spp. as potentially important human pathogens has been discussed (10, 36). Their similarity to Campylobacter might extend to infective capacity and the range and extent of the foodborne illness. The objective of this study was to determine the susceptibly of Arcobacter spp. to various environmental and food-related stress factors. Because this genus comprises species that are considered emerging human pathogens, it was important to determine the growth and survival capabilities of these organisms and the relationship to their potential presence and survival in contaminated foods.

MATERIALS AND METHODS

Preparation of inoculum. Human isolates of Arcobacter spp. (A. butzleri NADC 3556, 3257, 3494, 3539; A. cryaerophilus

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NADC 1A 3252 and 1B 3256) were obtained from Dr. Irene Wesley at the U.S. Department of Agriculture Agricultural Research Service–National Animal Disease Center Laboratory (Ames, Iowa) and stored at −70°C in Mueller-Hinton broth supplemented with 20% glycerol. Before use, they were subcultured onto 5% bovine blood agar plates (prepared with blood agar base with low pH Acumedia Manufacturers Inc., Baltimore, Md.; defibrinated bovine blood, Metro Medical Supply, Inc., Burlington, N.C.) and incubated for 36 to 48 h in a controlled gas atmosphere (10% CO₂, 5% O₂, 85% N₂) in a CO₂ water-jacketed incubator (model 3130, Forma Scientific, Marietta, Ohio). Isolated colonies of each culture were individually inoculated into liquid growth media (1:5 dilution with sterile distilled water) with 10 µl polystyrene loops (VWR Scientific, Westchester, Pa.). The medium used was Ellinghausen McCullough Johnson Harris (EMJH) PLM-5 medium, pH 7.35 (Intergen Inc., Purchase, N.Y.) supplemented with 1% Oxoryx (Oxoryx Inc., Mansfield, Ohio), which was dispensed into sterile 250-ml polystyrene tissue culture flasks (Becton Dickinson, Sparks, Md.) and incubated on an orbital shaker with gentle shaking (50 rpm) at 25°C (A. cryaerophilus) or 37°C (A. butzleri) for 30 h. This incubation period was selected on the basis of preliminary growth curve experiments that determined that target stationary-phase cells were obtained in this period. Cultures were centrifuged (8,000 × g, 25 min, 4°C, Sorvall RC-5B refrigerated centrifuge, DuPont Instruments, Newtown, Conn.), washed twice with 0.1 M phosphate-buffered saline (PBS; 0.85% NaCl, pH 7.4) and resuspended in PBS to yield a final concentration of 10⁸ CFU/ml. Prepared cultures were stored at 4°C until used, but for no more than 72 h.

**pH tolerance.** EMJH medium with 1% Oxoryx was distributed into a series of 500-ml flasks and adjusted aseptically to pH 3.5 to 8.0 at 0.5-unit intervals with 1 N HCl or 0.1 N NaOH. Portions from each flask (4 ml) were distributed into test tubes (80 by 12 mm) and inoculated with appropriately diluted cultures of A. butzleri or A. cryaerophilus to yield a final concentration of 10⁵ CFU/ml in each tube. Uninoculated controls were maintained for each pH level studied and were used as blanks for optical density measurements at 530 nm (OD₅₃₀). Tubes were incubated at 25 or 37°C, and the OD₅₃₀ was measured at time 0, 1, 2, 5, and 7 days. At each sampling time, a loopful of medium from each tube was spot-tested onto blood agar plates and inoculated into EMJH medium with Oxoryx to test for survival of cells near the growth limits of the strains. The experiment was repeated twice.

**NaCl tolerance.** The methods used were similar to the pH study, with the exception that appropriate amounts of a 35% NaCl solution (in deionized water adjusted to pH 7.35) were added to the flasks to provide a final NaCl content of 0.09 to 5.0% and inoculated tubes were incubated at 25 or 37°C for up to 96 h. It was determined from the manufacturer of EMJH medium that the base-level NaCl content in the medium was 0.09%. Thus, experimental media at higher NaCl contents received suitably adjusted supplementation with the 35% NaCl stock solution. A loopful of medium from each tube was spot tested onto blood agar plates and inoculated into EMJH medium with Oxoryx, as described in the pH study. The experiment was repeated twice.

**D-value determination in liquid medium.** Fifty microliters of PBS suspensions of A. butzleri 3257, 3556, and 3494 was filled by capillary action into 50-µl capillary tubes (Micropipets, VWR Scientific) that were sealed at both ends with a high-temperature flame from a National Air Gas torch (model 3A-B, Wale Apparatus Co., Hellertown, Pa.). For experiments at pH 5.5, the suspension of cells grown as described in the “Preparation of inoculum” section was centrifuged prior to use (8,000 × g, 25 min, 4°C) and resuspended in PBS adjusted to pH 5.5 to yield a final concentration of 10⁹ CFU/ml. The suspension was stored at 4°C until used, but for no more than 1 h. Filled capillary tubes were inserted into a plastic mesh covering on a test tube rack, so as not to be exposed to possible direct conductive heating effects of the base of the water bath. Test tube racks were placed in a circulating water bath (model M20B, Lauda Circulating water bath, Brinkmann Instruments, Westbury, N.Y.) at either 50, 55, or 60°C, and capillary tubes were removed at time intervals ranging from 2 s to 20 min, depending on the temperature used. These were immediately immersed in cooling water at 20°C for a period of 2 min. Capillary tubes were surface sterilized by dipping in 95% ethanol for 10 s, with any adhering ethanol rinsed off by swirling the capillary tubes in sterile distilled water. Unheated tubes were maintained as initial inoculum controls. The ends were broken off and the capillary tube was crushed with a sterilized glass rod into 4.95 ml of PBS. Appropriate dilutions were made, and aliquots were plated onto 5% bovine blood agar plates, which were incubated in the controlled gas mixture atmosphere for up to 48 h. Log counts obtained (CFU per milliliter) were plotted against time (minutes), and a linear regression was obtained, from which the D-value was calculated (negative reciprocal of the slope of the linear regression equation). Three replications of each experiment were carried out.

**D-value determination in ground pork.** Suspensions of A. butzleri 3257, 3556, and 3494 were prepared as above. On the basis of predetermined counts on blood agar plates, appropriate aliquots of the three cultures were mixed together to obtain a three-strain cocktail approximating 10⁹ CFU/ml, with equivalent counts (CFU per milliliter) of all three strains. Ground pork (75% lean) was obtained from a supermarket (HyVee Inc., West Des Moines, Iowa) and irradiated (30 kGy) at the Linear Accelerator Facility at Iowa State University (Ames, Iowa) to eliminate background microflora that were likely to confound the results. Frozen packages of irradiated pork were shipped to Athens, Ga., and stored at −18°C until used. As needed, portions of ground pork were thawed overnight at 4°C, mixed for homogeneity, and injected into 13-mm collagen sausage casings (Devro Teepak, Swansea, S.C.) with a sausage stuffer attached to a mixer (model K5-A, Kitchenaid Inc.). Individual 10-g portions of ground pork sausage were tied off. Arco bacter suspensions (1 ml) were injected into the center of each sausage portion with a 5-ml syringe. Inoculated sausages were inserted through a plastic wire mesh covering over a test-tube rack, which was placed in a circulating water bath at either 50 or 55°C. Come-up, hold, and cooling times were measured by a data recorder (model RD106, Omega Engineering, Stamford, Conn.), and sausage portions were removed at selected intervals for up to 45 min, depending on the temperature used. Each treated portion was quickly placed in a stomacher bag, 100 ml of PBS was added, and the sample was stomached (Tekmar model 400, Tekmar, Cincinnati, Ohio) for 2 min at normal speed. Aliquots of appropriate dilutions were plated on 5% bovine blood agar plates, which were incubated in a modified gas atmosphere (10% CO₂, 5% O₂, 85% N₂), and D-values were obtained as described above. Three replications of each experiment were carried out.

**Reduced temperature tolerance experiments.** Capillary tubes (50 µl) were filled with a suspension of A. butzleri 3556 prepared as outlined in the D-value section, placed in a circulating water bath at 50°C for 30 s or 1 min, and then immediately immersed in an ice-water or cold-water bath maintained at 4, 8, 12, or 16°C for 15 s, 30 s, 45 s, or 1 min. Capillary tubes were then
treated for recovery of cells as outlined above. The experiment was repeated twice.

RESULTS

The growth and survival responses of *A. butzleri* and *A. cryaerophilus* strains to varying pH, NaCl, and temperature influences were elucidated in these studies. Strains of *A. butzleri* and *A. cryaerophilus* grew in Oxyrase-supplemented EMJH medium between pH 5.5 and 8.0, with little if any growth below pH 5.0. The optimum pH for most *A. butzleri* strains was in the range of 6.0 to 7.0, whereas the optimum for *A. cryaerophilus* was pH 7.0 to 7.5 because the experimental inoculations reached a maximum cell density at these pH levels across the 5-day incubation period (Figs. 1A and 1B, 2A and 2B). Differences in cell densities at different pH levels were observed between the *A. butzleri* strains studied, with *A. butzleri* 3556 exhibiting the lowest cell density across all growth pH levels at both 25 and 37°C for the 2-day incubation period. On the other hand, *A. butzleri* 3539 achieved higher cell densities compared with the other *Arcobacter* strains studied at 37°C for the 2-day incubation period and at both temperature levels for the 5-day incubation period. At 2 days, *A. butzleri* strains were able to tolerate a lower pH (5.5) at 25°C, but not at 37°C. This additional tolerance was not exhibited at the 5-day incubation. The temperature dependence of growth at levels near the lower pH limits is also exhibited at 2 days, wherein a temperature of 25°C yielded higher cell numbers at pH 5.5 for all *A. butzleri* strains compared with 37°C. Also, for *A. butzleri* strains, although the lower temperature of 25°C did not initially (2 days; Fig. 1A versus 1B) appear to be as optimum as 37°C, on prolonged incubation (5 days; Fig. 2A versus 2B), these strains achieved higher numbers at 25°C. At both 25 and 37°C for an incubation period of either 2 or 5 days, growth of both strains of *A. cryaerophilus* was minimal, even when it did occur. This trend was observed through the entire period of experimentation with these strains. However, a temperature of 25°C and prolonged incubation appeared to favor the growth of both *A. cryaerophilus* 1A 3252 and 1B 3256.

In the presence of NaCl, the range of growth was between 0.09 and 3.0% and 0.09 and 3.5% NaCl for each *A. cryaerophilus* and *A. butzleri* strain studied (Tables 1 and 2). Individual strains exhibited varying growth and tolerance ranges over the 96-h study. Tolerance was noted as the occurrence of growth on subculture for primary exper-
FIGURE 2. Growth response of Arcobacter butzleri (A.b.) and Arcobacter cryaerophilus (A.c.) strains incubated in Ellinghausen McCullough Johnson Harris (EMJH) medium supplemented with Oxyrase at varying pH levels at 37°C (A) and 25°C (B) for 5 days. All values are representative of two replications.

TABLE 1. Response of Arcobacter butzleri (A.b.) and Arcobacter cryaerophilus (A.c.) strains to varying concentrations of NaCl at 25°C when incubated in Ellinghausen McCullough Johnson Harris (EMJH) medium (pH 7.3) supplemented with Oxyrase for 48 and 96 h; values are representative of two replications.

<table>
<thead>
<tr>
<th>Strains</th>
<th>%NaCl at 48 h</th>
<th>%NaCl at 96 h</th>
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<tbody>
<tr>
<td></td>
<td>Optimum growth</td>
<td>Max. growth</td>
</tr>
<tr>
<td>A.b. 3556</td>
<td>0.09</td>
<td>2.0</td>
</tr>
<tr>
<td>A.b. 3257</td>
<td>1.00</td>
<td>2.0</td>
</tr>
<tr>
<td>A.b. 3494</td>
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<td>2.0</td>
</tr>
<tr>
<td>A.b. 3539</td>
<td>0.09</td>
<td>2.0</td>
</tr>
<tr>
<td>A.c. 3252</td>
<td>0.09</td>
<td>2.0</td>
</tr>
<tr>
<td>A.c. 3256</td>
<td>0.09</td>
<td>2.0</td>
</tr>
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</table>

a A. cryaerophilus 3252 grew poorly, and results are not reported in the table.
b Maximum cell density (OD530) observed at these NaCl levels in EMJH medium with Oxyrase.
c Positive growth response at these NaCl levels in EMJH medium with Oxyrase.
d Positive growth response after subculture onto blood agar plates and in EMJH growth medium.
e No additional tolerance observed above growth level.
TABLE 2. Response of Arcobacter butzleri (A.b.) and Arcobacter cryaerophilus (A.c.) strains to varying concentrations of NaCl at 37°C when incubated in EllinghausenMcCullough Johnson Harris (EMJH) medium (pH 7.3) supplemented with Oxyrase for 48 and 96 h; values are representative of two replications

<table>
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<th>%NaCl at 96 h</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Optimum growth</td>
<td>Maximum growth</td>
</tr>
<tr>
<td>A. b. 3556</td>
<td>0.09</td>
<td>3.5</td>
</tr>
<tr>
<td>A. b. 3257</td>
<td>0.09</td>
<td>2.0</td>
</tr>
<tr>
<td>A. b. 3494</td>
<td>0.50</td>
<td>3.0</td>
</tr>
<tr>
<td>A. b. 3539</td>
<td>0.09</td>
<td>3.0</td>
</tr>
<tr>
<td>A. c. 3256</td>
<td>2.00</td>
<td>3.0</td>
</tr>
</tbody>
</table>

a A. cryaerophilus 3252 grew poorly and results are not reported in the table.
b Maximum cell density (OD530) observed at these NaCl levels in EMJH medium with Oxyrase.
c Positive growth response at these NaCl levels in EMJH medium with Oxyrase.
d Positive growth response after subculture onto blood agar plates and in EMJH growth medium.
e No additional tolerance observed above growth level.

immental inoculations not exhibiting growth as turbidity in OD530 measurements. Strains A. butzleri 3556 and 3539 and A. cryaerophilus 1B survived an NaCl concentration of 5.0% after 2 days at 25°C. These tolerance levels dropped to 4.0% NaCl after a 96-h incubation, with the exception of A. butzleri 3539, which continued to show high NaCl tolerance. The optimum NaCl level for A. cryaerophilus 1B 3256 was 0.5% to 1% after a 24-h incubation period (results not shown) and was 0.5% to 2% after 96 h. For the four A. butzleri strains, optimum NaCl levels ranged between 0.09 and 0.5% on the basis of the maximum population density that was observed at these levels.

Decimal reduction times (D-values) and z-values for the three A. butzleri strains studied are shown in Table 3. Figure 4 shows the tailing effect observed in the thermal inactivation studies when Leptospira medium (WCG Serum Products, Centerville, Utah) was used as the heating medium. Tailing was not observed when EMJH medium was used. It was observed that strains of A. butzleri varied slightly in their sensitivity to the lethal effects of the heating temperatures. The combination of heat and reduced pH brought about a decrease in the D-values. For strains 3556 and 3257, this reduction at pH 5.5 was 26 to 50% and 21 to 66%, respectively. However, for strain 3494, the pH effect was less because the D-values at pH 5.5 were 0 to 28% lower than those at pH 7.0. The z-values calculated from D-value observations were similar for all three strains of A. butzleri studied. At pH 5.5, the range was between 5.55 and 6.28°C, and at pH 7.3, the range was between 5.20 and 6.11°C. The decimal reduction time for the three-strain mixture of A. butzleri in heated ground pork at 50 and 55°C was found to be 18.51 and 2.18 min, respectively. The mean come-up times at 50 and 55°C were 4.25 and 4.11 min, respectively, whereas the mean cooling times from 50 and 55°C were 2 and 2.38 min, respectively.

Heating A. butzleri 3556 to 50°C for 30 s or 1 min, followed by immediate low-temperature treatment, decreased the numbers of surviving cells compared with a control receiving no low-temperature treatment (Fig. 3). This effect was more pronounced at lower temperatures of 4 and 8°C compared with 12 or 16°C and was observed in both sets of results. The cold shock effect was more a function of degree of low temperature used than the magnitude of heating time involved for the treatment times studied. A 3.2- to 4.0-log decrease in cell numbers at a cold shock temperature of 4°C was the maximum reduction observed, followed by a 2.27- to 3.34-log decrease at 8°C, a 1.31- to 2.73-log decrease at 12°C, and a 0.33- to 1.12-log decrease at 16°C. The reduction in cell numbers observed when heat was the only stress factor applied averaged 0.14 to 0.17 log cycles for 30 s or 1 min.

**DISCUSSION**

Arcobacter spp. have aroused interest only within the last two decades (34), largely because of their close phenotypic and genotypic relationship to Campylobacter spp., a somewhat elusive species in the laboratory, yet known to be responsible for a large number of cases of foodborne

TABLE 3. D-values (decimal reduction times, min ± SD) and z-values (°C) of three human isolates of Arcobacter butzleri in phosphate-buffered saline (PBS) at pH 7.3, and in PBS acidified with 1 N HCl (pH 5.5); these numbers are representative of three replications

<table>
<thead>
<tr>
<th>Strain no.</th>
<th>pH 5.5</th>
<th>pH 7.3</th>
<th>pH 5.5</th>
<th>pH 7.3</th>
<th>pH 5.5</th>
<th>pH 7.3</th>
<th>pH 5.5</th>
<th>pH 7.3</th>
</tr>
</thead>
<tbody>
<tr>
<td>3556</td>
<td>0.06 ± 0.001</td>
<td>0.12 ± 0.005</td>
<td>0.42 ± 0.01</td>
<td>0.76 ± 0.01</td>
<td>3.77 ± 0.23</td>
<td>5.12 ± 0.19</td>
<td>5.55</td>
<td>6.11</td>
</tr>
<tr>
<td>3257</td>
<td>0.03 ± 0.001</td>
<td>0.07 ± 0.003</td>
<td>0.30 ± 0.01</td>
<td>0.38 ± 0.02</td>
<td>1.97 ± 0.05</td>
<td>5.81 ± 0.33</td>
<td>5.61</td>
<td>5.20</td>
</tr>
<tr>
<td>3494</td>
<td>0.11 ± 0.005</td>
<td>0.10 ± 0.002</td>
<td>0.40 ± 0.03</td>
<td>0.56 ± 0.01</td>
<td>4.42 ± 0.52</td>
<td>5.31 ± 0.15</td>
<td>6.28</td>
<td>5.81</td>
</tr>
</tbody>
</table>
disease, especially in the developed world (29). Although substantial research data have been accumulated on the characteristics of Campylobacter spp., notably on the pathogenetic species Campylobacter jejuni and Campylobacter coli, research on Arcobacter spp. has centered around establishing its reservoirs in nature and its distribution within the food industry. This study has elucidated important characteristics of the genus, in particular, of strains of the primary human pathogen A. butzleri. Its similarity to Campylobacter warrants a comparison of characteristics between the two genera.

Much has been discussed of the role of campylobacters as VNCs (viable-but-nonculturable cells). This has been based on the rapid decline in cell numbers observed in starvation or reduced water activity environments (12), as in unchlorinated water or on poultry or beef surfaces (20). Similar observations were made in our experience of handling Arcobacter species, which experienced a rapid decline in cell numbers on the surface of 5% bovine blood agar plates, becoming unculturable on agar media transfers in about 3 to 4 weeks at 4°C. Subsequent inoculations into liquid growth media (EMJH) and prolonged incubation (for up to 1 week) resulted in resuscitation of the strains. Also, experimental inoculations of three A. butzleri strains in raw ground pork at levels of 4 to 6 log CFU/g, followed by incubation at 37°C for up to 72 h exhibited strain-dependent patterns. Although one strain showed neither growth nor survival in pork, the other two strains were able to survive, with no increase in cell numbers, at the highest inoculation level for periods of up to 72 h. Thus, the significance of Arcobacter spp. in foods and as potential pathogens might be related to their ability to survive under various environmental conditions, while not necessarily experiencing an increase in cell numbers.

Thermal tolerance is a characteristic not usually associated with campylobacters, in spite of the high growth range preference of the “thermophilic species” (13). Reported D-values at pH 7.0 in phosphate buffer of two human enteric C. jejuni strains (20) were in the range of 0.88 to 1.63 min at 50°C, whereas D-values of Campylobacter in foods ranged from 0.79 to 2.25 min at 57 to 55°C in cooked chicken, 0.21 to 13.3 min at 60 to 50°C in lamb cubes, and 0.62 to 5.9 min at 56 to 50°C in ground beef (20). From our experiments, Arcobacter strains studied were somewhat more thermostolerant, with D-values ranging from 0.03 to 5.81 min at 60 to 50°C in phosphate buffered saline and 2.18 to 18.51 min at 55 to 50°C in ground pork. This apparent enhanced resistance factor, coupled with its published ability to survive and grow under aerobic conditions, makes Arcobacter spp. significant as potential foodborne pathogens when compared with available literature on Campylobacter studies. A combination of heat and acid pH (5.5) decreased heat resistance of A. butzleri, although one strain appeared to be less susceptible (A. butzleri 3494) to these combined effects compared with the others (A. butzleri 3556 and 3257). pH 5.5 was chosen because one of the primary reservoirs of arcobacters is swine/raw pork products, which have pH values of 5.5 to 5.6 (5, 11). The closest comparisons available are the D-values of Campylobacter jejuni in poultry scald water, which were 0.4 and 8.72 min at 52°C at pH 4.0 and 6.0, respectively (20).

An interesting feature noted in our studies was the apparent effect of preexperimental growth medium conditions on defining the thermotolerance of A. butzleri. Early determination of D-values was conducted with the use of Leptospira medium. Subsequent to nonavailability of this medium, experiments were conducted with EMJH medium. Although cultures grown in both media were washed twice
in PBS before the experiments, a consistent tail region was observed in survivor curves at 50°C for strain 3556 when the WCG Serum products medium was used for preexperimental growth of cells compared with the EMJH medium. It is possible that presence or levels of one or more ingredients might be a contributing factor in this observation. The main differences between the two media are the use of Tween 80 and copper sulfate in EMJH, as opposed to Tween 20 and no copper sulfate in the WCG Serum products formulation. Withell (37) conducted an in-depth study on the variation in shape of time-survivor curves and has noted the existence of lag times or tailing in survivor curves. Although it is well known that a difference in the age of cells can bring about a curve shape variation, it is possible that certain media ingredients can also emphasize cell thermal variations, as seen in our study. Tailing has been observed with a number of different microorganisms and experimental techniques. Humpheson et al. (17) reported reproducible biphasic thermal survivor curves with higher resistance in tail subpopulations in Salmonella Enteritidis PT4 cultures studied at 60°C. The authors report that de novo protein synthesis of heat shock proteins is responsible for the observed tailing in Salmonella Enteritidis. Moats et al. (25) have stated that the variations observed in tail populations were of physiological rather than of genetic origin because subcultures of heat-resistant cells exhibited no more heat resistance than the parent cultures.

A successive combination of two potential stress-inducing effects (i.e., heat followed by cold shock) might work synergistically to bring about a large reduction in microcol cell numbers. This effect was observed distinctly on heating A. butzleri to 50°C, followed by low-temperature treatments of 4 and 8°C. Yogasundram and Shane (38) mention the susceptibility of Campylobacter spp., wherein freezing reduces cell numbers by 1 to 2 log cycles on the surface of poultry carcasses. Although these synergistic effects have not been tested “in situ” with Arcobacter spp., spraying carcasses with chilled water or disinfectant sprays after scalding would be a cheap, effective intervention method. Humphrey and Cruickshank (18) described a related study on C. jejuni strains wherein altered resistance is subsequent to treatment with chemical antibacterial agents was observed following either freezing at −20°C or heating at 50°C. Populations surviving these sublethal treatments were found to be more sensitive to rifampicin and sodium deoxycholate. The authors suggest that a loss of barrier properties of the bacterial outer membrane was responsible for the responses noted.

Studies conducted in our laboratory also noted growth of some A. butzleri strains in EMJH medium at 10°C and pH 6.5 to 8.0 at a minimum incubation period of 7 days. Earlier studies have reported the lower growth limit for Arcobacter spp. to be 15°C (35). Additionally, we observed that all A. butzleri isolates studied were able to survive prolonged incubation at −20°C (6 months, EMJH medium) or −70°C (up to 24 months, Mueller Hinton medium with 20% glycerol) with, depending on the strain, a 0- to 1.5-log decrease in cell numbers.

Arcobacter spp. were found to have a pH growth range of 5.5 to 8.0, with an optimum for growth of pH 6.0 to 7.5, depending on the species and strain. Some strains can tolerate pH 5.0, especially at nonoptimal growth temperatures (25°C), for up to 2 days. In comparison, the reported pH growth range for Campylobacter is 4.9 to approximately 9.0, with an optimum of pH 6.5 to 7.5 (20). The consistently low maximum cell numbers attained by A. cryaerophilus strains compared with A. butzleri strains, as observed in the pH study, is probably a reflection of the inability of this species to attain sufficiently high numbers in natural and food environments. This is perhaps the reason why reported isolation rates of A. butzleri strains in the literature are higher.

Arcobacter spp. can grow at NaCl levels up to 3.0 or 3.5%, depending on the species and strain of concern. Some strains survive NaCl levels of up to 5.0%, especially at nonoptimal growth temperatures (25°C) for periods of up to 2 days. The reported optimum NaCl concentration for growth of Campylobacter spp. was 0.5%, with a maximum of 1.5% (20). Thus, in comparison, Arcobacter spp. can grow in and tolerate higher NaCl concentrations. Our studies indicate that strains of Arcobacter are microaerophilic species with higher NaCl and temperature resistance compared with Campylobacter spp. and with a pH growth and survival level comparable to that of Campylobacter. It is possible that under the same environmental conditions, arcobacters would be capable of enhanced survival against competing campylobacters and could be responsible for foodborne diseases that are mistakenly identified as Campylobacter related. Further research is necessary to determine the interacting effects of environmental stresses that have been noted in this paper, with a view to designing a cost-effective strategy for control of campylobacter-like organisms in food products.

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REFERENCES


