Survival and Growth of Acid-Adapted and Unadapted Salmonella in and on Raw Tomatoes as Affected by Variety, Stage of Ripeness, and Storage Temperature

LARRY R. BEUCHAT* AND DAVID A. MANN

Center for Food Safety and Department of Food Science and Technology, University of Georgia, 1109 Experiment Street, Griffin, Georgia 30223-1797, USA

MS 08-051: Received 25 January 2008/Accepted 19 March 2008

ABSTRACT

Consumption of raw round and Roma tomatoes has been associated with outbreaks of salmonellosis. A study was done to determine whether survival and growth of Salmonella in and on tomatoes is affected by variety of tomato, stage of ripeness, and storage temperature. The influence of acid adaptation of cells and site of inoculation on survival and growth was studied. Salmonella grew in stem scar and pulp tissues of round, Roma, and grape tomatoes stored at 12 and 21°C but not in those tomatoes stored at 4°C. Survival and growth was largely unaffected by variety and stage of ripeness at the time of inoculation. The pathogen did not grow on the skin of grape tomatoes stored at 4, 12, and 21°C. Survival and growth of Salmonella inoculated into stem scar and pulp tissues of round and Roma tomatoes were unaffected by exposure of cells to an acidic (pH 4.75) environment before inoculation. Results emphasize the importance of preventing contamination of tomatoes with Salmonella at all stages of ripeness, regardless of variety or previous exposure of cells to an acidic environment.

Several outbreaks of salmonellosis have been associated with consumption of raw tomatoes (4–8, 14, 22). Contamination of tomatoes by Salmonella can occur at several points in the preharvest and postharvest continuum of production, processing, distribution, and preparation in food service and home environments (2, 18). Analysis of wash water collected from tomatoes obtained from retail markets revealed that as high as 55% of healthy ripe tomatoes and 64% of soft-rotted tomatoes were positive or presumptive positive for Salmonella (28). Salmonella was confirmed to be present in tissue samples of 4 of 6 healthy tomatoes and 7 of 17 rotten tomatoes. Growth of Salmonella in tomato tissues can be enhanced by coinfection with molds (24, 25, 29).

Once Salmonella attaches to the surface of tomatoes or infiltrates tissues, it can persist and may grow throughout the expected shelf life. The pathogen can grow in tomato tissue at pH 4.1 (1) and at refrigeration temperatures (21, 24). Temperature and relative humidity affect the extent to which Salmonella cells attach to ripe tomatoes (15). Populations of Salmonella Montevideo on the surface of mature green tomatoes stored at 10°C for 18 days have been reported to not change significantly (32). Populations of the same serotype inoculated on the surface of green tomatoes did not change significantly when tomatoes were treated with 100 ppm of ethylene at 100% relative humidity and 20°C for 6 days (17). Several other Salmonella serotypes have been observed to persist on the surface of green and ripe (red) tomato fruits (21) and on tomato leaves (17) and stems (11). Survival appears to be serotype dependent (11, 21). Salmonella Montevideo appears to survive or persist longer than other Salmonella serotypes on and in tomatoes. Depending on temperature, relative humidity, atmospheric gas composition, and other factors, Salmonella on the surface of tomatoes may grow (21, 32) or die (9, 12, 31).

Asplund and Nurmi (1) were the first to report that Salmonella can grow in diced raw red tomatoes. Salmonella Typhimurium, Salmonella Infantis, and Salmonella Enteritidis grew at 22°C to populations exceeding 10⁸ CFU/g. Salmonella Baildon was observed to grow well in diced red tomatoes stored at 21 or 30°C (27). Death occurs on diced tomatoes stored at 4°C (20, 27). Salmonellae are known to be able to grow on sliced tomatoes (26) and in the stem scar and pulp tissues of ripe tomatoes (9, 24–26).

Survival and growth characteristics of Salmonella as affected by tomato variety and stage of ripeness have been given little research attention. Yuk et al. (31) reported that Roma tomatoes had a significantly higher pH than round tomatoes, but survival of salmonellae in wounds and on the surface was not affected by variety. The behavior of acid-adapted Salmonella in and on tomatoes has likewise been given only meager research attention. Weissinger et al. (27) observed that tolerance of Salmonella Baildon to an agar medium at pH 4.5 was not influenced by the pH of tomato juice (4.8 or 5.8) or broth (pH 7.2) in which it had been grown. Acid-adapted cells of Salmonella Montevideo inoculated into homogenized Roma tomatoes are more resistant, however, than are unadapted cells to electron beam irradiation (16).

*Author for correspondence. Tel: 770-412-4740; Fax: 770-229-3216; E-mail: lbeuchat@uga.edu.
The objective of the study reported here was to determine whether survival and growth of *Salmonella* in and on raw tomatoes is affected by the variety of tomato (round, Roma, and grape), stage of ripeness, and storage temperature. The influence of acid adaptation of cells and site of inoculation on survival and growth also was studied.

**MATERIALS AND METHODS**

**Bacterial cultures.** Five *Salmonella enterica* serotypes were used: Agona (isolated from alfalfa sprouts), Baildon (from a patient in an outbreak of salmonellosis associated with diced tomatoes), Gaminara (from orange juice), Michigan (from a cantaloupe-associated outbreak of salmonellosis), and Montevideo (from a patient in a tomato-associated outbreak of salmonellosis). All serotypes were grown at 37°C for 24 h in tryptic soy broth with 0.25% glucose (Difco, Becton Dickinson, Sparks, Md.) supplemented with 50 μg/ml nalidixic acid (TSBN). Cultures of each serotype were combined with sterile glycerol (85:15, vol/vol, culture-glycerol) and stored at −20°C until used.

**Tomatoes.** Three varieties of tomatoes (*Lycopersicon esculentum* L.) distinguished by shape and size (round, Roma, and grape) were used. Tomatoes at various stages of ripeness were purchased at retail and wholesale produce markets in the Atlanta, Ga., area. Color classification (green, breakers, turning, pink, light red, and red) was subjectively judged according to a U.S. Department of Agriculture tomato ripeness color chart (23). Tomatoes were held at 12 or 21°C, depending on the experiment, for 16 to 18 h immediately preceding inoculation with *Salmonella*.

**Survival and growth of *Salmonella* in round and Roma tomatoes.** The first series of experiments was done to determine the effect of temperature on survival and growth of *Salmonella* in pulp (radial pericarp) and stem scar tissues of round and Roma tomatoes initially at the turning and/or pink stages of ripeness. Cells of five *S. enterica* serotypes grown in TSBN at 37°C for 24 h were separately transferred (ca. 10 μl) to 10 ml of commercially manufactured tomato juice (pH 4.3) supplemented with 50 μg/ml nalidixic acid (TJN) and adjusted to pH 4.8 by adding sterile 0.1 M NaOH. Cultures were incubated at 37°C and transferred (ca. 10 μl) to TJN at 48-h intervals. Equal volumes of 48-h cultures were combined to give a five-serotype mixture containing approximately equal populations of each serotype. This suspension of cells adapted to the acidic TJN environment was serially diluted in sterile deionized water to give an inoculum containing ca. 4 log CFU/ml.

The pulp and stem scar tissue of round and Roma tomatoes at 12 or 21°C were inoculated via a syringe with 20 μl of the five-serotype suspension. A site on the skin approximately half way between the stem scar tissue and the blossom end was selected for inoculation of the pulp tissue. A circle was drawn on the skin around the inoculation site to facilitate visual detection of the site for later removal of tissue for pH measurement and microbiological analysis. The syringe needle was inserted in the pulp or stem scar tissue at a depth of ca. 5 mm before injecting the suspension. Inoculated tomatoes were placed in a ventilated box and stored for up to 27 days at 12 or 21°C and 15 and 36% ± 6% relative humidity, respectively. Microbiological analysis and subjective observations of color were made after storage of tomatoes at 12°C for 0, 3, 6, 10, 14, and 27 days and at 21°C for 0, 3, 6, 10, and 14 days. The pH of item scar tissue and pulp at the site of inoculation and of un inoculated pulp in each tomato also was measured.

**Survival and growth of acid-adapted and unadapted cells in round tomatoes.** A second series of experiments was done to compare survival and growth characteristics of acid-adapted and unadapted *Salmonella* cells in stem scar tissue and pulp of green and yellow and red round tomatoes. Each serotype was grown in TSBN containing no glucose (TSBN−G, initial pH of 7.1) and TSBN supplemented with 1% glucose (TSBN+G, initial pH 7.1) at 37°C for 24 h. After three consecutive transfers (ca. 10 μl into 10 ml of TSBN−G and TSBN+G) at 24-h intervals, cells were collected by centrifugation (8,000 × g) and resuspended in 10 ml of sterile deionized water. Equal volumes of suspensions of cells of the five serotypes grown in TSBN−G (cells not adapted to acidic environment) were combined to give a five-serotype mixture containing approximately equal numbers of each serotype. The same procedure was used to prepare a five-serotype mixture of cells grown in TSBN+G, i.e., acid-adapted cells. Suspensions were serially diluted to give inocula containing ca. 2 log CFU/ml. Stem scar and pulp tissues of tomatoes at 4, 12, and 21°C were inoculated as described above with acid-adapted and unadapted cells. Microbiological analyses, observations of tomato color, and pH measurements were recorded after storage of tomatoes at 4, 12, and 21°C for 0, 3, 6, and 10 days.

**Survival and growth of acid-adapted and unadapted cells in diced tomatoes.** Roma tomatoes at the light red to red stages of ripeness were adjusted to 12°C. Tomatoes were surface sanitized by immersing and continuously agitating for 2 min in sterile 0.05 M potassium phosphate buffer (pH 6.8, 4°C) initially containing 200 μg/ml free chlorine. The tomato–chlorinated buffer ratio was 1:3 (wt/vol). Tomatoes were drained, and stem ends were removed with a knife and discarded. Tomatoes were diced with a knife into pieces ca. 1 by 1 by 1 cm in size. Portions of the diced tomatoes (2,000 g) were immersed in 4,000 ml of sterile 0.21% CaCl₂ solution or sterile deionized water (control) at 4°C, continuously agitated for 30 s, and drained. Treatment of raw diced tomatoes with CaCl₂ enhances retention of tissue fluids, thereby promoting retention of texture. This process is commonly applied to commercially prepared diced tomatoes intended for use in salads, fresh-tomato salsas, and Mexican-style dishes such as tacos.

Portions (450 g) of tomatoes were inoculated with 5 ml of suspensions of acid-adapted or unadapted *Salmonella* prepared as above and diluted in sterile water. Samples (25 g) in Stomacher 400 bags (Seward Medical, London, UK) stored at 4, 12, and 21°C for 0 (within 30 min after inoculation), 3, 6, and 9 days were analyzed for the presence (by enrichment) and populations of *Salmonella*.

**Survival and growth of *Salmonella* in and on grape tomatoes.** Experiments were done to determine the survival and growth characteristics of *Salmonella* in the stem scar and pulp tissues and on the skin surface of grape tomatoes. A five-serotype mixture of cells grown in TSBN−G, i.e., not adapted to an acidic environment, was diluted in sterile water to give a population of ca. 1.76 log CFU/ml (57 CFU/ml). Stem scar and pulp tissues were inoculated with 20 μl of suspension as described above for round and Roma tomatoes, except that separate tomatoes were used to study the behavior of *Salmonella* at each inoculum site. The skin surface of another set of grape tomatoes was inoculated by distributing 20 μl of suspension at five or six locations, and inoculated tomatoes were placed on an elevated rack in a biological safety cabinet for 2 h at 21°C to evaporate the water in the inoculum. Three tomatoes (19.5 ± 2.5 g) inoculated at one of three sites (stem scar, pulp, or skin surface) were placed in vented Ziploc bags (17.7 by 19.6 cm; Dow Chemical Co., Midland,
Mich.) and stored at 4, 12, and 21°C and 58, 15, and 38% relative humidity, respectively, for up to 14 days before analyzing them for the presence and populations of Salmonella. Each sample consisted of a composite of three tomatoes inoculated at the same site.

**Microbiological analysis.** Stem scar and pulp tissues of inoculated tomatoes were analyzed for the presence (by enrichment) and population of Salmonella within 30 min (0 day) and after 3 to 13 days of storage at 4, 12, or 21°C for up to 27 days. Samples (2 g) of inoculated stem scar tissue and pulp (ca. 1.5 cm surface diameter, 1.2 cm deep) of round and Roma tomatoes were removed with a sterile stainless steel scalpel. Each sample was deposited in a Stomacher 80 bag (Seward Medical). The stem scar tissue was macerated with a small mallet. To bags containing stem scar tissue or pulp, 18 ml of sterile 0.05 M potassium phosphate buffer (pH 6.8) was added, and the mixture was pummeled in a stomacher (Seward Medical) at medium speed for 2 min.

For experiments focused on determining survival and growth characteristics of Salmonella grown in tomato juice followed by inoculation into round and Roma tomatoes, pulmed homogenates were surface plated (0.25 ml in quadruplicate and 0.1 ml in duplicate) on tryptic soy agar supplemented with 50 μg/ml nalidixic acid (TSAN; Difco, Becton Dickinson) and xylose lysine desoxycholate (XLD) agar (Difco, Becton Dickinson). Samples serially diluted in sterile 0.1% peptone were also surface plated (0.1 ml in duplicate) on TSAN and XLD agar. Presumptive Salmonella colonies formed on plates incubated at 37°C for 24 h were counted. Cells from selective colonies were subjected to a Salmonella latex agglutination test (Oxoid, Basingstoke, UK) and an API 20E biochemical assay (bioMérieux Vitek, Hazelwood, Mo.) for confirmation.

Homogenates (ca. 20 ml) of stem scar tissue or pulp tissue in phosphate buffer were enriched by adding 20 ml of 2% lactose broth (Difco, Becton Dickinson) supplemented with 100 μg/ml nalidixic acid (2X LBN) and incubating the mixture at 37°C for 24 h. If no presumptive Salmonella colonies appeared on TSAN or XLD agar plates, a loopful of the enriched sample was streaked on XLD agar and incubated at 37°C for 24 h. Cells from colonies presumptive for Salmonella were subjected to confirmation tests as described above.

Formation of colonies by background microflora other than Salmonella on XLD agar occasionally interfered with the detection of presumptive Salmonella colonies. In all experiments subsequent to the first series of experiments involving determination of survival and growth characteristics of Salmonella grown in tomato juice and inoculated into round and Roma tomatoes, XLD agar was replaced with bismuth sulfate agar (Difco, Becton Dickinson) supplemented with 50 μg/ml nalidixic acid (BSAN) as a selective direct plating medium.

Diced Roma tomatoes were analyzed for the presence (by enrichment) and population of Salmonella within 30 min (0 days of storage) of inoculation and after storing at 4, 12, and 21°C for 3, 6, and 10 days. Samples (25 g) in Stomacher 400 bags were combined with 225 ml of lactose broth supplemented with 50 μg/ml nalidixic acid (LBN) and pummeled in a stomacher at medium speed for 2 min. Samples were diluted and incubated as described above, streaked onto BSAN, and incubated at 37°C for 24 h before cells from presumptive colonies were subjected to confirmation tests.

Grape tomatoes were analyzed for the presence (by enrichment) and population of Salmonella within 30 min (0 days of storage) of inoculation and after storing at 4, 12, and 21°C for 3, 6, 10, and 14 days. Each sample (three tomatoes, ca. 20 g) in a Stomacher 400 bag was combined with 80 ml of sterile phosphate buffer and pummeled in a stomacher at medium speed for 2 min. Undiluted homogenates and homogenates serially diluted in sterile 0.1% peptone water were surface plated on BSAN. To the remaining homogenate, 100 ml of 2X LBN was added, and the mixture was incubated at 37°C for 24 h. Plates on which undiluted and diluted homogenates were spread were incubated at 37°C for 24 to 27 h before presumptive Salmonella colonies were counted. If no presumptive Salmonella colonies developed on BSAN, enriched samples were streaked on BSAN. Plates were incubated at 37°C for 24 h before examining for presumptive Salmonella colonies. Confirmation of presumptive colonies was done as described above.

**Measurement of pH.** The pH of stem scar tissue and pulp was measured with an accuCap electrode (glass body, gel filled, spear tip; Fisher Scientific, Fair Lawn, N.J.) and a pH meter (Denver Instrument Company, Denver, Colo.). The pH of tomato juice, broths, and agars was measured with a flat-surface electrode.

**Statistical analysis.** All experiments were replicated two or three times, and duplicate samples representing each test parameter combination in each replicate trial were analyzed with a general linear model on SAS software (version 8.0, SAS Institute, Cary, N.C.). The least significant difference test was used to determine significant differences (α = 0.05) in mean populations of Salmonella detected in tomatoes subjected to various treatments and storage conditions.

**RESULTS AND DISCUSSION**

Undiluted and diluted homogenates of tomatoes were surface plated on TSAN and either XLD agar or BSAN throughout the study. In several instances, significantly lower (α = 0.05) counts were obtained on XLD agar and BSAN than on TSAN. Rarely were counts on TSAN significantly lower than counts on XLD agar or BSAN. Only the data obtained using TSAN as an enumeration medium are reported.

Changes in populations of Salmonella in stem scar and pulp tissues in round and Roma tomatoes that were inoculated at the turning to the pink stages of ripeness and stored at 12 and 21°C are shown in Figure 1. The initial population (day 0) was 0.08 (±0.02) log CFU/g of tissue at the inoculation site. The relative humidities of the environments in which tomatoes were stored were 15 and 36% at 12 and 21°C, respectively, which is lower than the 75 to 95% range used in commercial tomato storage but closer to relative humidities of environments to which tomatoes are exposed in retail and food service settings. Over time, populations of Salmonella increased significantly in stem scar and pulp tissues of tomatoes stored at both temperatures. Higher populations (4.9 to 8.4 log CFU/g) were reached at 21°C than at 12°C (3.3 to 4.9 log CFU/g) in tomatoes stored for 14 and 27 days, respectively. This finding is in agreement with those of other studies in which Salmonella was found in stem scar tissues (32) and pulp tissues (25, 26) of raw tomatoes. In contrast, Yuk et al. (31) reported that Salmonella did not grow in the wounds of round or Roma tomatoes. Differences in stage of ripeness of tomatoes and storage temperatures may have affected the growth characteristics of Salmonella reported in these studies.
Although *Salmonella* appeared to grow more rapidly in round tomatoes than in Roma tomatoes, at least toward the end of the storage period, there were only a few significant differences in counts obtained from three replicate trials with two varieties of tomatoes stored at the same temperature for the same time (Fig. 1). Yuk et al. (31) also observed that variety of tomato did not significantly influence survival and growth of *Salmonella*. Within the same variety of tomato, rates of growth of *Salmonella* were largely unaffected by the type of tissue into which the pathogen was inoculated.

The initial pH values (day 0) of pulp tissues in round and Roma tomatoes at the turning to the pink stages of ripeness were 4.16 ± 0.06 and 4.28 ± 0.10, respectively. Increases to pH 4.87 and 4.81, respectively, occurred in round and Roma tomatoes stored at 12°C for 14 days; the pH of round and Roma tomatoes stored at 21°C increased to 4.56 and 4.68 within 3 and 6 days, respectively. This trend of increase in pH as tomatoes ripen has been reported by others (10, 13, 19, 30). Contrary to these findings, Brecht et al. (3) found that the pH of six of eight tomato varieties was higher in mature green fruit than in table-ripe fruit. In our study, tomatoes stored at 21°C ripened more quickly than did those stored at 12°C. The red stage of ripeness was reached within 3 days at 21°C and within 6 days at 12°C.

*Salmonella* grown in tomato juice (pH 4.8) was used to inoculate tomatoes in the studies described above. In the second set of experiments, *Salmonella* cultures grown in TSBN+G and TSBN−G were used as inocula; these cells had pH values 4.75 and 7.07, respectively. The goal was to determine whether acid-adapted cells (grown in TSBN+G) behaved differently than unadapted cells (grown in TSBN−G) upon inoculation into stem scar and pulp tissues of round green tomatoes followed by storage for up to 10 days at 12°C and 21°C. Results are shown in Figure 2. Populations of *Salmonella* in stem scar tissue of tomatoes stored at 12°C did not change significantly during the 10-day storage period, regardless of previous exposure of cells to an acidic environment. In pulp tissue, significantly higher numbers of acid-adapted *Salmonella* cells were detected in tomatoes stored at 12°C for 6 and 10 days than in tomatoes stored at 21°C.
stored for 0 and 3 days. When tomatoes were stored at 21°C, populations of cells increased in both types of tissue, regardless of acid adaptation preceding inoculation. Higher populations were reached in pulp tissue than in stem scar tissue. As observed in the first series of studies, *Salmonella* populations grew more rapidly at 21°C than at 12°C. In only one combination of test parameters (stem scar tissue of tomatoes stored at 12°C for 3 days) was the population of *Salmonella* significantly affected by previous exposure to an acidic environment.

The color of tomatoes changed during the 10-day storage period. Tomatoes stored at 12°C ripened to the turning or pink stage, whereas tomatoes stored at 21°C were light red to red. Concurrent with the ripening process, the initial pH of the stem scar tissue (4.80) and pulp tissue (4.08) increased significantly to 5.32 to 5.93 and 4.44 to 4.74, respectively, during storage of tomatoes at 12°C. Increases in the stem scar tissue and pulp tissue to 5.00 to 5.66 and 4.55 to 4.67, respectively, were observed in tomatoes stored at 21°C. Overall, there is no strong evidence that survival and growth of *Salmonella* in tissues of round tomatoes inoculated at the green stage of ripeness are affected by the level of adaptation of cells to acid before inoculation.

Figures 3 and 4 show the changes in populations of *Salmonella* in light red to red round and Roma tomatoes, respectively, as affected by site of inoculation (stem scar or pulp tissue), type of *Salmonella* cell in inoculum (acid adapted or unadapted), and storage temperature (12 or 21°C) for up to 10 days. Tomatoes were initially (day 0) at light red and red stages of ripeness. With the exception of stem scar tissue in round tomatoes inoculated with acid-adapted cells and stored at 12°C, significant increases in populations of *Salmonella* occurred in round tomatoes, regardless of type of tissue, type of cells, or storage temperature during the 10-day storage period (Fig. 3). As with
FIGURE 5. Number of Salmonella cells isolated from diced red Roma tomatoes treated with 0.21% CaCl₂, inoculated with acid-adapted and unadapted cells at two populations (low inoculum, 0.88 to 0.99 log CFU/g; high inoculum, 2.88 to 2.99 log CFU/g), and stored at 12°C (top) and 21°C (bottom) for up to 10 days. Within each cell type (acid adapted or unadapted), inoculum level (low or high), and storage temperature (12 or 21°C), bars with the same letter are not significantly different.

FIGURE 6. Number of Salmonella cells isolated from the stem scar and pulp tissues of grape tomatoes at the red stage of ripeness. Tomatoes were inoculated (1.75 log CFU/g) with cells that had not been exposed to an acid environment and were stored at 12 and 21°C for up to 14 days. Within each tissue type (stem scar or pulp) and storage temperature, bars with the same letter are not significantly different. An asterisk indicates that the number of Salmonella cells detected in pulp tissue was significantly higher than the number isolated from the stem scar tissue of tomatoes stored for 6 days at 12°C or 3 days at 21°C.

round green tomatoes (Fig. 2), higher populations were reached at 21°C than at 12°C. Overall, higher populations were reached in light red to red round tomatoes (Fig. 3) than in green round tomatoes (Fig. 2). This may be attributed in part to the higher initial pH and perhaps sugar content of light red to red tomatoes versus green tomatoes.

In only two instances (stem scar tissue of round tomatoes stored at 12°C for 10 days and pulp tissue of round tomatoes stored at 12°C for 3 days) did the type of cell in the inoculum result in significant differences in populations (Fig. 3). In both cases, a significantly higher number of Salmonella cells was detected in tissues inoculated with acid-adapted cells than in tissues inoculated with cells not adapted to an acidic environment.

Populations of Salmonella detected in stem scar tissue of Roma tomatoes inoculated with acid-adapted cells and stored at 12°C for up to 10 days did not increase significantly (Fig. 4). However, populations detected in three replicate trials did significantly increase in tomatoes subjected to all other test parameters. With the exception of stem scar tissue of tomatoes inoculated with cells not adapted to acid and stored for 6 days at 21°C, populations of Salmonella detected at a given storage time were not significantly affected by cell type. As with round light red to red tomatoes (Fig. 3) and round green tomatoes (Fig. 2), Salmonella grew to higher numbers at 21°C than at 12°C.

Studies were done to determine survival and growth characteristics of Salmonella in diced tomatoes stored at 4, 12, and 21°C for up to 10 days (Fig. 5). Acid-adapted and unadapted cells were inoculated into diced red Roma tomatoes (pH 4.30) at low (0.88 to 0.99 log CFU/g) and high (2.88 to 2.99 log CFU/g) inoculum levels. Treatment of diced tomatoes with 0.21% CaCl₂ did not have a significant effect on the behavior of Salmonella. Only data for the tomatoes treated with CaCl₂ are shown in Figure 5.

Populations of Salmonella in diced tomatoes stored at 4°C did not change significantly during a 10-day period (data not shown). The number of Salmonella cells in low-inoculum diced tomatoes stored at 12°C did not change significantly during storage, regardless of preexposure of cells to an acid environment. In tomatoes receiving the high inoculum and stored at 12°C and in tomatoes receiving either a low or high inoculum and stored at 21°C, populations increased during 10 days of storage. Higher populations were reached in tomatoes stored at 21°C than in tomatoes stored at 12°C. Growth of cells was unaffected by previous exposure to acid pH.

Survival and growth characteristics of Salmonella in stem scar and pulp tissues and on the skin surface of grape tomatoes stored at 4, 12, and 21°C (58, 15, and 38% relative humidity, respectively) for up to 14 days were determined. The inoculum population was 1.75 log CFU per inoculation
site. Cells not exposed to acidic pH were used to prepare inocula. Changes in populations of *Salmonella* in stem scar and pulp tissues of tomatoes stored at 12 and 21°C are shown in Figure 6. In a survey of red round and Roma tomatoes collected from several retail markets, we found that mean pH values were 4.37 and 4.40, respectively, and mean soluble solids ("Brix value") for round and Roma tomatoes were 4.1 and 3.9, respectively. In our study, the initial pH of grape tomatoes was 4.67 and the "Brix value" was 6.2; these values are higher than those for red round and red Roma tomatoes and would be presumed to provide a more favorable environment for growth of *Salmonella*. Compared with growth rates in light red to red round and Roma tomatoes (Fig. 4) and diced red Roma tomatoes (Fig. 5), however, these differences in initial pH and "Brix did not have a marked effect.

Significant increases in populations of *Salmonella* occurred in stem scar and pulp tissues of grape tomatoes stored at 12 and 21°C. Higher numbers were reached in tomatoes stored at 21°C than in tomatoes stored at 12°C. With two exceptions (tomatoes stored for 6 days at 12°C or 3 days at 21°C), the number of *Salmonella* cells detected in stem scar and pulp tissues after a given storage time was not significantly different. *Salmonella* was detected by enrichment in tissues and on the skin surface of tomatoes stored at 4°C for up to 10 to 14 days, but growth did not occur (data not shown). The pathogen did not grow on the skin of inoculated grape tomatoes nor was it detected on tomatoes stored at 12 and 21°C for 14 days or longer.

Results of these studies show that *Salmonella* can grow in pulp tissue of round, Roma, and grape tomatoes stored at 12 and 21°C and that the variety and stage of ripeness at the time of inoculation have little effect on survival and growth. Previous exposure of *Salmonella* to an acidic environment did not consistently influence the ability of cells to survive and grow in tomatoes. These observations emphasize the importance of preventing contamination of tomatoes with *Salmonella* at all stages of ripeness, regardless of variety or previous exposure of the pathogen to an acidic environment.

REFERENCES


