

CHEMICAL SANITIZING AGENTS AND SPOILAGE BACTERIA ON FRESH BROILER CARCASSES

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Primary Audience: Quality Assurance Personnel, Directors of Research, Microbiologists, Sanitation Managers

SUMMARY

The effect of sanitizers on spoilage bacteria associated with poultry is not well known, and this study investigated this phenomenon. *Pseudomonas fluorescens*, *Pseudomonas putida*, *Pseudomonas fragi*, and *Shewanella putrefaciens* were collected from spoiled broiler carcasses. The following sanitizers in three replicate trials (Rep) were applied to the isolates: sodium hypochlorite (SH), quaternary ammonium (Quat), lactic acid (LA), trisodium phosphate (TSP), hydrogen peroxide (HP), and Timsen (TN), a novel compound containing a Quat derivative in combination with urea. SH eliminated growth of *P. fluorescens*, *P. putida*, and *P. fragi*, but did not consistently kill *Shewanella putrefaciens* at concentrations ≤ 50 ppm. Quat did not consistently eliminate any of the spoilage bacteria analyzed. At levels of 5% or above, LA eliminated or inhibited all spoilage isolates. At 0.5%, TSP eliminated pseudomonads, whereas a level of 1% or higher was required to prevent *S. putrefaciens* growth. HP was an effective sanitizer at 0.1%. Few spoilage bacteria multiplied in the presence of 10 ppm TN. *S. putrefaciens* grew when exposed to 10 ppm or 100 ppm TN in Reps 1 and 2, respectively. All of the sanitizers tested, except Quat, prevented growth of the pseudomonads. In general, *S. putrefaciens* was more resistant to sanitizers than were the pseudomonads.

Key words: Inhibition, poultry, sanitizers, spoilage bacteria

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DESCRIPTION OF PROBLEM

In the United States, most poultry production is concentrated in the Southeast, and thus, products must be transported long distances to reach their final destination. The majority of this poultry is sold fresh and is highly perishable. Shelf life of fresh poultry is a function of the number of spoilage bacteria on the product's surface initially as well as of the amount of time that the product has been held under refrigeration prior to arrival at its final destination. Psychrotrophic spoilage bac-

teria are associated with the feathers and feet of the live bird, the water supply in the processing plant, chill tanks, and other plant equipment, but these bacteria are not usually found in the intestines of the live bird [1, 2]. Therefore, to produce poultry with maximum shelf life, plant cleaning and sanitation must effectively destroy spoilage bacteria on processing plant equipment.

Russell *et al.* [3] reported that the most common off-odor producing bacteria isolated from spoiled broiler chicken carcasses were *Shewanella putrefaciens*, *Pseudomonas*

fluorescens, *P. fragi*, and *P. putida*. Halleck *et al.* [4] reported that *Pseudomonas fluorescens* constituted approximately 80% of the bacterial species on meat in the latter part of storage. Thus, these species of bacteria are of particular concern when optimizing sanitation strategies for extending shelf life.

Previous research has demonstrated that spoilage bacteria may be resistant to commonly used commercial sanitizers. Stone and Zottola [5] reported that *Pseudomonas fragi* attached to stainless steel were able to survive the combined effects of cleaning with 2500 ppm alkali detergent for 7 min, cleaning with 500 ppm of an acidic detergent for 3 min, and sanitizing with sodium hypochlorite (SH) at 100 ppm for 3 min. Wirtanen and Mattila-Sandholm [6] demonstrated that SH at a level of 0.1% was able to decrease *Pseudomonas fluorescens* counts at 24, 48, 72, and 144 hr in meat soup; however, 0.1% SH increased *P. fluorescens* counts in milk after 48 hr. SH completely inhibited growth of *P. aeruginosa* at concentrations as low as 50 ppm after 300 sec of exposure [7].

Hingst *et al.* [8] showed that *Pseudomonas putida* and *P. fluorescens* were resistant to quaternary ammonium (Quat) compounds. *Pseudomonas aeruginosa* was not inhibited by 50 or 200 ppm basic Quat compound at pH levels ranging from 7.29 to 8.80 after 300 sec of exposure [7]. Ouattara *et al.* [9] found that growth of *Pseudomonas fluorescens* was inhibited in acetic acid at 0.1 and 0.2%, propionic acid at 0.1 and 0.2%, lactic acid (LA) at 0.3%, and citric acid at 0.2 and 0.3% for a period of 24 hr. After 24 hr, the bacteria were able to multiply in the presence of the sanitizer. However, *P. fluorescens* was not inhibited by LA at concentrations below 0.3% or by citric acid at 0.1%. Mountney and O'Malley [10] found that LA at a concentration of 0.275% was least effective among the organic acids as a means of extending shelf life of fresh poultry. In addition, longer exposure to 1,3-dichloro-2,2,5,5-tetramethylimidazolidin-4-one (DC) and 3-chloro-4,4-dimethyl-2-oxazolidinone (I) sanitizer [11] was required to inactivate *P. fluorescens* than was required for *Salmonella enteritidis* or *Salmonella typhimurium*. Most studies have focused on commonly used chemical sanitizers; however, there are some novel sanitizers that have gained popularity. Currently, there is little or no data on the effect

of Quat, trisodium phosphate (TSP), hydrogen peroxide (HP), or Timsen (TN) on specific spoilage bacteria from poultry.

The purpose of this study was to determine the effect of commonly used and novel commercial sanitizing agents on bacteria associated with spoiled poultry. The hypothesis was that some species of spoilage bacteria may be resistant to sanitizing agents.

MATERIALS AND METHODS

SPOILAGE BACTERIAL ISOLATES

Broiler carcasses were collected from processing plants in Georgia, Arkansas, California, and North Carolina. Carcasses were individually bagged in sterile polyethylene bags (3000 cc O₂ at 22.8°C/m²/24 hr at 1 atm) and held on ice until arrival at the laboratory. Carcasses were allowed to spoil under controlled conditions at 3±0.5°C for 15 days. After spoilage, the carcasses were rinsed with 100 mL of sterile deionized water. The rinse fluid was diluted to 10⁻⁶, 10⁻⁷, and 10⁻⁸ using a sterile 1% solution of Bacto-peptone (Difco Laboratories, Detroit, MI), and 1 mL of the diluent was spread onto duplicate plate count agar (PCA-Difco) plates. Plates were incubated at 25°C for 48 hr. Each isolate was assayed for Gram reaction, cytochrome oxidase activity, and production of catalase, in addition, each isolate was identified using either the Vitek (bioMérieux Vitek, Inc., Hazelwood, MO), Biolog (Biolog, Inc., Hayward, CA), or Micro-ID (Organon Teknika Corporation, Durham, NC) rapid identification methods. *Pseudomonas fluorescens*, *Pseudomonas putida*, *Pseudomonas fragi*, and *Shewanella putrefaciens* isolates from spoiled carcasses were collected and used in this study.

SANTIZERS

All stock solutions containing sanitizing chemicals were prepared by adding sanitizers individually to bottles containing 100 mL of 0.45% sterile saline to achieve the desired concentration. The concentration of the stock solution of each sanitizer and the final treatment concentrations of each sanitizer were as follows: SH stock 5.25%, treatment groups 30, 50, and 70 ppm; Quat stock (Antec Quat 800) 13.2%, treatment groups 10, 20, and 30 ppm; LA stock 85% (Baker, Phillipsburg, NJ), treatment groups, 1, 5, and 10%; TSP stock 98%

(Sigma Chemical, St. Louis, MO), treatment groups 0.5, 1, and 2%; HP stock 3% (Baker), treatment groups 0.1, 0.5, and 1%; Timsen (TN) (crystals-United Promotions, Inc., Atlanta, GA), treatment groups 10, 25, and 100 ppm. TN is a novel compound containing a Quat derivative in combination with urea.

ADDITION OF BACTERIA

To determine the effect of a sanitizer on each isolate, *Pseudomonas fluorescens*, *P. putida*, *P. fragi*, and *Shewanella putrefaciens* were individually placed into brain heart infusion broth (BHI-Difco) at 25°C for 24 hr. Each actively growing culture was diluted by placing 1 mL of the culture into 99 mL of sterile Bacto-peptone (Difco). A 1 mL portion of this mixture was placed into 99 mL of saline containing each sanitizer and allowed to remain for 1 hr to allow the sanitizer enough time to damage the bacteria. One mL of this mixture was placed into 99 mL of sterile BHI (Difco), which acts as a growth medium for conducting impedance assays. Electrical measurements (impedance) have been previously used as a means of determining the minimum inhibitory concentration of sanitizing agents for inhibiting the growth of psychrotrophic bacteria [12]. Impedance microbiology is based on electrical changes that occur in growth medium as bacteria multiply and produce metabolites. As metabolites accumulate in the medium, they eventually reach a "threshold level," and the impedance shift that is produced by the accumulation of the metabolites is sufficient to be detected. This impedance shift generally occurs as bacteria reach a level of 10^6 CFU/mL. The time required for bacteria to produce an impedance shift is called the detection time (DT), and it is a function of the medium, competing microorganisms, and the incubation temperature. Thus, if impedance detection times are increased by the addition of chemical sanitizer, then the sanitizer had an inhibitory effect on the growth of the spoilage bacterium. On the other hand, if no detection time was achieved in 48 hr, then growth of the organism was considered completely inhibited. One mL of this mixture was placed into a Bactometer module well in duplicate. Impedance was monitored using the Bactometer Microbial Monitoring System M128 (bioMérieux Vitek, Inc., Hazelwood, MO) at 25°C for 48 hr.

STATISTICAL ANALYSES

The experimental design was a $3 \times 4 \times 6$ of replication, spoilage bacteria, and sanitizer. All microbiological analyses were conducted in duplicate. Data were analyzed after averaging the duplicates. Results were analyzed using the General Linear Models (GLM) procedure of SAS software [13]. Treatment means were separated using Fisher's Least Significant Difference option of SAS software [13]. Upon testing treatment, replication, and treatment \times replication interaction, replication was found to have a significant effect, but treatment \times replication interaction was not significant ($P < .05$). Therefore, pooled error was used to separate treatment means. All values reported as significant were analyzed at the $\alpha = .05$ level.

RESULTS AND DISCUSSION

Differences in inhibition were observed between replicates (Rep). For each replicate, different bacterial isolates were used to make bacterial suspensions. Thus, the differences observed between replicates may be attributed to the individual resistance of each bacterial isolate to each sanitizer.

Impedance detection times (hr) are presented for pure cultures of *Pseudomonas fluorescens*, *P. putida*, *P. fragi*, and *Shewanella putrefaciens* in brain heart infusion broth (BHI) with 0, 30, 50, or 70 ppm SH (Table 1), 0, 10, 20, or 30 ppm Quat (Table 2), 0, 1, 5, or 10% LA (Table 3), 0, 0.5, 1, or 2% TSP (Table 4), 0, 0.1, 0.5, or 1% HP (Table 5), or 0, 10, 20, or 100 ppm TN (Table 6). SH completely eliminated the growth of *Pseudomonas fluorescens* at 30 ppm or greater, except in Rep 1. SH eliminated the growth of *P. putida* and *P. fragi* at 30 ppm or higher, except for *P. fragi* in Rep 2, which grew at 70 ppm SH. *Shewanella putrefaciens* (formerly *Pseudomonas putrefaciens*) [14] tolerated levels of SH as high as 30 ppm in Rep 1 and 50 ppm in Rep 2; however, in Rep 3, 30 ppm SH was sufficient to eliminate its growth. These data suggest that SH is an effective sanitizing agent for *P. fluorescens*, *P. putida*, and *P. fragi*, but may not be sufficient for inhibiting the growth of *Shewanella putrefaciens* at low concentrations (30 ppm).

Unlike SH, Quat allowed *Pseudomonas fluorescens* and *P. fragi* to multiply in its presence, even at levels as high as 30 ppm.

TABLE 1. Impedance detection times (hr) for pure cultures of *Pseudomonas fluorescens*, *P. putida*, *P. fragi*, and *Shewanella putrefaciens* in brain heart infusion broth (BHI) with 0, 30, 50, or 70 ppm sodium hypochlorite^A

| SANITIZER CONCENTRATION | PSEUDOMONAS FLUORESCENS | | | PSEUDOMONAS PUTIDA | | | PSEUDOMONAS FRAGI | | | SHEWANELLA PUTREFACIENS | | |
|-------------------------|-------------------------|---------|---------|--------------------|---------|---------|-------------------|------------------------|---------|-------------------------|------------------------|----------|
| | Rep 1 | Rep 2 | Rep 3 | Rep 1 | Rep 2 | Rep 3 | Rep 1 | Rep 2 | Rep 3 | Rep 1 | Rep 2 | Rep 3 |
| 0 ppm | 7.8±0.0 ^b | 9.1±0.0 | 8.9±0.0 | 11.8±0.0 | 7.7±0.0 | 7.8±0.0 | 11.2±0.6 | 8.4±0.0 ^b | 8.5±0.0 | 9.8±0.04 ^b | 13.5±0.0 ^c | 15.2±0.0 |
| 30 ppm | 36.7±0.05 ^a | - | - | - | - | - | - | - | - | 29.4±0.05 ^a | 37±0.02 ^a | - |
| 50 ppm | - | - | - | - | - | - | - | - | - | - | 38.2±0.01 ^a | - |
| 70 ppm | - | - | - | - | - | - | - | 32.6±0.05 ^a | - | - | - | - |

^A - indicates no growth in 48 hr.

^{a-c} Means within columns with no common superscript differ significantly ($P < .05$).

TABLE 2. Impedance detection times (hr) for pure cultures of *Pseudomonas fluorescens*, *P. putida*, *P. fragi*, and *Shewanella putrefaciens* in brain heart infusion broth (BHI) with 0, 10, 20, or 30 ppm quaternary ammonia^A

| SANITIZER CONCENTRATION | PSEUDOMONAS FLUORESCENS | | | PSEUDOMONAS PUTIDA | | | PSEUDOMONAS FRAGI | | | SHEWANELLA PUTREFACIENS | | |
|-------------------------|-------------------------|------------------------|-----------------------|-----------------------|-----------------------|-----------------------|-----------------------|-----------------------|-----------------------|-------------------------|----------|----------|
| | Rep 1 | Rep 2 | Rep 3 | Rep 1 | Rep 2 | Rep 3 | Rep 1 | Rep 2 | Rep 3 | Rep 1 | Rep 2 | Rep 3 |
| 0 ppm | 7.8±0.0 ^b | 9.1±0.0 ^c | 8.9±0.0 ^d | 11.8±0.0 ^b | 7.7±0.0 ^d | 7.8±0.0 ^b | 11.2±0.6 ^b | 8.4±0.0 ^c | 8.5±0.0 ^c | 9.8±0.4 ^c | 13.5±0.0 | 15.2±0.0 |
| 10 ppm | 11.8±0.8 ^a | 10.4±0.2 ^{bc} | 11.4±0.8 ^c | - | 11.6±0.2 ^c | 18.4±0.2 ^a | - | 10.8±0.2 ^b | 15.2±0.2 ^b | 10.6±0.4 ^c | - | - |
| 20 ppm | 13.6±0.2 ^a | 13.8±1.2 ^a | 15.8±0.8 ^b | 14.8±0.2 ^a | 14.6±0.0 ^b | - | 17.0±0.4 ^a | 14.2±0.0 ^a | 17.4±0.4 ^a | 25.4±0.4 ^a | - | - |
| 30 ppm | 13.4±1.2 ^a | 12.8±1.0 ^{ab} | 18.8±0.2 ^a | - | 17.8±0.0 ^a | - | - | 14.2±0.0 ^a | 17.8±0.8 ^a | 13.4±0.8 ^b | - | - |

^A - indicates no growth in 48 hr.

^{a-d} Means within columns with no common superscript differ significantly ($P < .05$).

TABLE 3. Impedance detection times (hr) for pure cultures of *Pseudomonas fluorescens*, *P. putida*, *P. fragi*, and *Shewanella putrefaciens* in brain heart infusion broth (BHI) with 0, 1, 5, or 10% lactic acid^A

| SANTIZER CONCENTRATION | PSEUDOMONAS FLUORESCENS | | | PSEUDOMONAS PUTIDA | | | PSEUDOMONAS FRAGI | | | SHEWANELLA PUTREFACIENS | | |
|------------------------|-------------------------|---------|---------|--------------------|---------|---------|-------------------|---------|---------|-------------------------|------------------------|-----------------------|
| | Rep 1 | Rep 2 | Rep 3 | Rep 1 | Rep 2 | Rep 3 | Rep 1 | Rep 2 | Rep 3 | Rep 1 | Rep 2 | Rep 3 |
| 0% | 7.8±0.0 | 9.1±0.0 | 8.9±0.0 | 11.8±0.0 | 7.7±0.0 | 7.8±0.0 | 11.2±0.6 | 8.4±0.0 | 8.5±0.0 | 9.8±0.4 | 13.5±0.0 ^c | 15.2±0.0 ^a |
| 1% | - | - | - | - | - | - | - | - | - | - | 45±0.05 ^a | 11.6±0.2 ^b |
| 5% | - | - | - | - | - | - | - | - | - | - | 44.6±0.05 ^b | - |
| 10% | - | - | - | - | - | - | - | - | - | - | - | - |

A₋ indicates no growth in 48 hr.
^{a-c}Means within columns with no common superscript differ significantly (P < .05).

TABLE 4. Impedance detection times (hr) for pure cultures of *Pseudomonas fluorescens*, *P. putida*, *P. fragi*, and *Shewanella putrefaciens* in brain heart infusion broth (BHI) with 0, 0.5, 1, or 2% trisodium phosphate^A

| SANTIZER CONCENTRATION | PSEUDOMONAS FLUORESCENS | | | PSEUDOMONAS PUTIDA | | | PSEUDOMONAS FRAGI | | | SHEWANELLA PUTREFACIENS | | |
|------------------------|-------------------------|---------|---------|--------------------|---------|---------|-------------------|---------|---------|-------------------------|----------|------------------------|
| | Rep 1 | Rep 2 | Rep 3 | Rep 1 | Rep 2 | Rep 3 | Rep 1 | Rep 2 | Rep 3 | Rep 1 | Rep 2 | Rep 3 |
| 0% | 7.8±0.0 | 9.1±0.0 | 8.9±0.0 | 11.8±0.0 | 7.7±0.0 | 7.8±0.0 | 11.2±0.6 | 8.4±0.0 | 8.5±0.0 | 9.8±0.4 ^b | 13.5±0.0 | 15.2±0.0 ^a |
| 0.5% | - | - | - | - | - | - | - | - | - | - | - | 15.1±0.05 ^a |
| 1% | - | - | - | - | - | - | - | - | - | 25.6±0.0 ^a | - | - |
| 2% | - | - | - | - | - | - | - | - | - | - | - | - |

A₋ indicates no growth in 48 hr.
^{a,b}Means within columns with no common superscript differ significantly (P < .05).

TABLE 5. Impedance detection times (hr) for pure cultures of *Pseudomonas fluorescens*, *P. putida*, *P. fragi*, and *Shewanella putrefaciens* in brain heart infusion broth (BHI) with 0, 0.1, 0.5, or 1% hydrogen peroxide^A

| SANTIZER CONCENTRATION | PSEUDOMONAS FLUORESCENS | | | PSEUDOMONAS PUTIDA | | | PSEUDOMONAS FRAGI | | | SHEWANELLA PUTREFACIENS | | |
|------------------------|-------------------------|---------|---------|--------------------|-----------------------|---------|-------------------|---------|---------|-------------------------|-----------------------|----------|
| | Rep 1 | Rep 2 | Rep 3 | Rep 1 | Rep 2 | Rep 3 | Rep 1 | Rep 2 | Rep 3 | Rep 1 | Rep 2 | Rep 3 |
| 0% | 7.8±0.0 | 9.1±0.0 | 8.9±0.0 | 11.8±0.0 | 7.7±0.0 ^b | 7.8±0.0 | 11.2±0.6 | 8.4±0.0 | 8.5±0.0 | 9.8±0.4 | 13.5±0.0 ^b | 15.2±0.0 |
| 0.1% | - | - | - | - | 23.4±0.0 ^a | - | - | - | - | - | 43±0.0 ^a | - |
| 0.5% | - | - | - | - | - | - | - | - | - | - | - | - |
| 1% | - | - | - | - | - | - | - | - | - | - | - | - |

^A - indicates no growth in 48 hr.

^{a,b} Means within columns with no common superscript differ significantly ($P < .05$).

TABLE 6. Impedance detection times (hr) for pure cultures of *Pseudomonas fluorescens*, *P. putida*, *P. fragi*, and *Shewanella putrefaciens* in brain heart infusion broth (BHI) with 0, 10, 25, or 100 ppm Timsen^A

| SANTIZER CONCENTRATION | PSEUDOMONAS FLUORESCENS | | | PSEUDOMONAS PUTIDA | | | PSEUDOMONAS FRAGI | | | SHEWANELLA PUTREFACIENS | | |
|------------------------|-------------------------|---------|---------|--------------------|-----------------------|---------|-------------------|---------|---------|-------------------------|------------------------|----------|
| | Rep 1 | Rep 2 | Rep 3 | Rep 1 | Rep 2 | Rep 3 | Rep 1 | Rep 2 | Rep 3 | Rep 1 | Rep 2 | Rep 3 |
| 0 ppm | 7.8±0.0 | 9.1±0.0 | 8.9±0.0 | 11.8±0.0 | 7.7±0.0 ^b | 7.8±0.0 | 11.2±0.6 | 8.4±0.0 | 8.5±0.0 | 9.8±0.4 ^b | 13.5±0.0 ^c | 15.2±0.0 |
| 10 ppm | - | - | - | - | 21.2±0.6 ^a | - | - | - | - | 26.4±0.2 ^a | 31.8±0.05 ^b | - |
| 25 ppm | - | - | - | - | - | - | - | - | - | - | - | - |
| 100 ppm | - | - | - | - | - | - | - | - | - | - | 45±0.05 ^a | - |

^A - indicates no growth in 48 hr.

^{a-c} Means within columns with no common superscript differ significantly ($P < .05$).

P. putida were inhibited at a concentration of 30 ppm Quat, except in Rep 2. *Shewanella putrefaciens* did not grow in 10 ppm Quat in Reps 2 and 3; however, in Rep 1, it multiplied well in the presence of 30 ppm Quat. Although Quat is one of the most commonly used surface contact sanitizers in the poultry industry, it would not be recommended for insuring the destruction of spoilage bacteria on equipment surfaces based on these results.

Organic acids have been shown to be effective for reducing pathogenic bacteria and for extending the shelf life of fresh and fully cooked poultry [15, 16, 17]. All species of spoilage bacteria were inhibited in the presence of LA, except *Shewanella putrefaciens*. *S. putrefaciens* multiplied in the presence of 1% LA in Reps 2 and 3, and 5% in Rep 2; however, the DT in the presence of LA at 5% or greater was greatly extended, indicating that the lag time to initiation of multiplication of the bacteria was extended. LA at levels of 5% or above appeared to reduce the presence of spoilage bacteria.

TSP at levels as low as 0.5%, completely eliminated the growth of all spoilage bacteria analyzed except for *Shewanella putrefaciens*.

Although its growth was inhibited, as evidenced by a significantly higher DT, *S. putrefaciens* was able to multiply in the presence of 1% TSP in Rep 1. TSP at a level of 0.5% in Rep 3 did not significantly affect the growth of *S. putrefaciens*. TSP at very low concentrations appears to be effective for eliminating the growth of the pseudomonads, whereas to control the growth of *S. putrefaciens*, levels of 1% or higher may be required.

HP at levels as low as 0.1%, eliminated growth of all species of spoilage bacteria, except *P. putida* in Rep 2 and *S. putrefaciens* in Rep 2. Although *P. putida* and *S. putrefaciens* were able to multiply in 0.1% HP, their growth was significantly inhibited (DT's were significantly higher than controls).

None of the pseudomonads were able to multiply in the presence of 10 ppm TN except *P. putida* in Rep 2, which was greatly inhibited. *S. putrefaciens*, although inhibited, was able to proliferate when exposed to 10 ppm TN in Rep 1, or 10 and 100 ppm TN in Rep 2. Overall, TN appears to be much more effective at low concentrations than Quat compounds at eliminating the growth of spoilage bacteria.

CONCLUSIONS AND APPLICATIONS

1. Sodium hypochlorite, lactic acid, trisodium phosphate, hydrogen peroxide, and Timsen at low concentrations were excellent for eliminating *Pseudomonas fluorescens*, *P. putida*, and *P. fragi*.
2. Quaternary ammonia may be inappropriate for sanitizing equipment surfaces as a means of removing or destroying spoilage bacteria.
3. *S. putrefaciens* appeared to be resistant to all sanitizers except quaternary ammonium. In this respect, *S. putrefaciens* differs from the pseudomonads, which are not as resistant to the sanitizers.
4. Timsen was more effective at low concentrations than quaternary ammonium at eliminating the growth of spoilage bacteria.

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