

Salmonella Contamination Associated with Bacterial Soft Rot of Fresh Fruits and Vegetables in the Marketplace

ABSTRACT

Wells, J. M., and Butterfield, J. E. 1997. *Salmonella* contamination associated with bacterial soft rot of fresh fruits and vegetables in the marketplace. *Plant Dis.* 81:867-872.

Wash water from 66% of 401 samples of fresh fruits and vegetables collected in the marketplace and affected by bacterial soft rot were positive for suspected strains of *Salmonella*, i.e., black, hydrogen sulfide-positive colonies on Salmonella-Shigella agar incubated for 24 h at 37°C. By comparison, 30% of 402 healthy samples were positive. Incidence of suspected *Salmonella* in broth enrichment cultures was 59% in 533 soft rotted samples and 33% in 781 healthy samples. Thirty percent of 166 representative strains of suspected *Salmonella*, selected at random from 20 different commodities, were confirmed to be *Salmonella* by physiological and serological tests. Adjusting incidence values accordingly, *Salmonella* contamination was potentially present in at least 18 to 20% of soft rotted samples and in 9 to 10% of healthy samples. Wash water from 120 paired healthy and soft rotted fruits and vegetables contained an average of 1.0×10^5 and 3.7×10^6 CFU/ml, respectively, of suspected *Salmonella*—a ratio of 1:37. Average concentrations of suspected *Salmonella* in enrichment cultures of healthy and soft rotted samples were 7.5×10^7 and 2.7×10^9 CFU/ml, respectively, also in the ratio of 1:37. Fresh potato, carrot, and pepper disks coinoculated with the soft rot bacterium *Erwinia carotovora* and with *Salmonella typhimurium*, and incubated for up to 72 h at room temperature, contained approximately 10 times the concentration of *S. typhimurium* as did disks inoculated with *Salmonella* alone. Disks coinoculated with *Pseudomonas viridiflava* and *S. typhimurium* contained approximately three times the *Salmonella* populations as disks inoculated with *Salmonella* alone.

Contamination of horticultural products by fecal coliforms is well documented and is recognized as a potential public health problem (13,14). Occasional reports of multistate outbreaks of salmonellosis in the United States associated with contaminated fresh fruits and vegetables have coincided with increased consumption of fresh produce in recent years due to changing consumer preferences, greater selections, wider distribution, and year-round availability (16). Recent outbreaks have been associated with *Salmonella chester* and *S. poona* on cantaloupes from the lower Rio Grande area in Texas (10), with *S. javania* and *S. montevideo* on tomatoes from North and South Carolina (9,27) and with *Salmonella* sp. on alfalfa sprouts (24). In foreign countries, as well as in the United States,

Salmonella outbreaks have been associated with consumption of celery, watercress, watermelon, lettuce, cabbage, and raw salad vegetables (13,14). As an indicator of enteric bacteria, *Salmonella* is commonly isolated from horticultural crops or from wash waters. In Italy, Ercolani found *Salmonella* in wash water from 68% of lettuce and 72% of fennel samples tested (8). In the United States, Rude et al. cultured *Salmonella* from 4 of 50 vegetables sampled (22). In Spain, Garcia-Villanova-Ruiz et al. detected *Salmonella* in 7.5% of market vegetables sampled (12), and incidence on various vegetable commodities has been reported in England (19), Egypt (23), Iraq (1), and Italy (11).

Although research and epidemiological data outline broad features of a problem, there are many obscure details relating to the causes of *Salmonella* contamination. Also unclear is the relationship between the incidence of indicator bacteria and actual clinical cases of salmonellosis, and specific factors associated with produce marketing and food preparation that aggravate the problem. The present research describes one possible source of contamination: the association between *Salmonella* and bacterial soft rot, one of the most common postharvest diseases of horticultural crops.

Bacterial soft rot is a leading cause of postharvest losses of potatoes (4), tomatoes

(6), peppers (7), lettuce (5), and other fresh fruits and vegetables in the marketplace. It is caused by a group of plant pathogens, harmless to humans, that includes *Erwinia carotovora* (subsp. *carotovora* and *atro-septica*), pectolytic *Pseudomonas fluorescens* (*P. marginalis*), and *P. viridiflava* (18). Pectolytic breakdown of affected tissues results in softening, liquefaction, and exudates that can spread bacteria over commodities in bulk storage or display, contaminate food-handling equipment, and protect bacteria from the environment (25,26).

E. carotovora, the most common member of the soft rotting bacterial complex, is a member of the *Enterobacteriaceae*, which includes *Salmonella* and other enteric bacteria of importance to public health. They can be soilborne, and they share ecological niches such as soft rotted plant tissues where liquefaction provides a rich bacteriological medium. *Salmonella* and *Erwinia* can be taxonomically separated by several key biochemical properties, but the most useful and distinguishing traits are their optimum growth temperatures and pathogenicity (3). This report specifically examines the association and interaction of the two in the context of bacterial soft rot disease of fresh fruits and vegetables.

MATERIALS AND METHODS

Collection of samples in the marketplace. Samples of 48 different fruits and vegetables, listed in Table 1, were collected in local supermarkets in Somerset and Middlesex counties, New Jersey, on a periodic basis between 1992 and 1995. Approximately twice a month, healthy specimens were randomly selected from retail displays. Healthy samples were those free of blemishes, wounds, or decayed areas. Soft rotted samples, as they were available, were obtained from materials culled by store personnel and from displays. Soft rotted samples were those with water-soaked or macerated lesions, with or without bacterial exudation, characteristic of early bacterial soft rot development (25). When matched samples were obtained, the healthy sample was collected from the vicinity of the soft rotted sample. Samples with advanced bacterial soft rot or collapsed tissues, or suspected of mixed bacterial-fungal infections, were not collected.

Preparation of washes and isolation protocol. Depending on the specific com-

modity, 10- to 600-g samples were washed with agitation for 30 min in 500 ml of sterile water. Healthy samples usually constituted an entire bunch of leafy herbs or greens (100 to 450 g), half to whole heads of lettuce and umbellifers depending on size (200 to 500 g), one or up to six of the larger fruits and vegetables (edible portions only, 300 to 600 g), and 6 to 18 of the smaller commodities such as snap beans, mushrooms, radishes, and cherry tomatoes (200 to 450 g). Soft rotted samples were washed as single whole items (200 to 600 g), excised portions (10 to 60 g), or in the case of leafy vegetables, as infected leaves

or portions of leaves (10 to 100 g). When paired samples were tested, the soft rotted portion of a sample was excised and tested as a subsample against the remaining healthy portion.

Samples were tested for possible (i.e., suspected) *Salmonella* by a modification of the method of Poelma et al. (21) designed (i) to process a large number of samples, (ii) to compare healthy and diseased samples, and (iii) as a preliminary screen for lactose-negative H₂S⁺ strains, a category that generally encompasses most *Salmonella* strains except for *S. paratyphi* A and some strains of *S. choleraesuis* (3). Wash

water (1 ml) was diluted 10-fold in selenite cystine enrichment broth (Difco Laboratories, Detroit, MI) and incubated at 37°C for 24 h, and 0.1 ml was surface-streaked on Salmonella-Shigella (SS) agar (Difco) by the technique described by Krieg (17) in 60-mm petri plates. In addition, for comparative purposes, 0.1 ml of wash water was streaked directly on SS agar without enrichment. SS plates were incubated at 37°C and examined at 24 and 48 h for black or black-centered (i.e., H₂S⁺) colonies.

Sixty field samples were used for a comparison of the modified method just

Table 1. Percent healthy and soft rotted fruits and vegetables positive for black, hydrogen sulfide-positive colonies (suspected *Salmonella*) in wash and in enrichment broth samples

Commodity	Healthy samples ^a				Soft rotted samples ^a			
	Wash ^b		Enriched ^c		Wash ^b		Enriched ^c	
	Samples (no.)	Positive (%)	Samples (no.)	Positive (%)	Samples (no.)	Positive (%)	Samples (no.)	Positive (%)
Alfalfa sprouts	5	100	5	100	7	100	7	86
Arrugula	10	40	10	20	11	82	11	100
Basil	8	25	8	25	11	55	11	46
Beans, snap	5	20	20	50	5	80	8	100
Bean sprouts	6	100	6	83	6	100	6	83
Beet, greens	7	14	10	10	9	78	13	62
Broccoli	8	12	8	0	7	71	7	86
Broccoli, rabe	10	40	10	30	10	50	11	64
Cabbage	10	20	10	20	9	56	10	30
Calabaza	11	54	11	45	9	78	9	67
Cantaloupe	6	67	17	47	7	57	8	87
Carrot	10	40	13	46	9	67	11	45
Cauliflower	8	12	16	12	8	50	14	36
Celery	5	0	42	24	7	43	10	30
Chicory	11	27	11	0	10	60	10	50
Cilantro	10	10	10	30	11	91	11	64
Collard greens	10	30	10	30	9	89	9	78
Cucumber	11	55	21	67	11	91	19	90
Dill	8	37	8	50	8	63	8	38
Escarole	8	50	10	20	7	86	8	87
Fennel	12	25	12	33	10	60	10	30
Kale	10	10	10	20	8	88	8	75
Lettuce, iceberg	7	0	24	8	7	0	21	10
Lettuce ^d , leaf	14	0	53	19	17	47	17	41
Mushroom	6	0	11	18	6	33	6	50
Onion, dry	11	0	11	9	9	33	9	33
Onion, green	5	60	15	20	8	50	8	25
Parsley	10	20	10	20	9	33	9	78
Parsnip	8	50	8	25	8	50	8	37
Pepper, bell type	18	39	66	35	18	72	58	66
Pepper, cubanelle	8	25	15	27	7	86	9	89
Pepper, jalapeño	10	20	10	50	9	89	9	89
Pepper, long hot	10	30	10	20	8	25	8	37
Potato ^e	11	91	13	100	9	100	10	100
Radish	9	0	10	30	10	70	10	70
Spinach	19	16	58	26	19	90	33	76
Squash ^f	7	14	18	61	6	83	7	86
Swiss chard	7	43	10	20	8	63	10	90
Tomato	11	55	83	40	11	64	37	41
Tomato, cherry	7	14	15	47	6	17	7	29
Tomato, plum	6	0	34	41	8	12	9	33
Turnip	10	20	10	10	8	75	8	50
Vegetables, mixed	8	38	8	50	12	75	12	67
Watercress	11	27	11	45	9	89	9	33
Totals ^g	402	30.3	781	32.9	401	65.8	533	59.3

^a Approximately 10- to 600-g portions of healthy or soft rotted tissues, depending on the commodity, washed in 500 ml of water for 30 min.

^b Wash plated directly on Salmonella-Shigella (SS) agar and incubated for 24 h at 37°C.

^c One ml of wash enriched in selenite cystine broth for 24 h at 37°C, then plated on SS agar and incubated for 24 h at 37°C.

^d Including Romaine lettuce.

^e Data combined for round red, round white, and russet potatoes.

^f Data combined for yellow and zucchini squash.

^g Totals and final averages based on data for aggregated samples.

described with the standard isolation protocol of enrichment in selenite cystine and in tetrathionate broth, and streaking on Hektoen Enteric (HE), xylose lysine desoxycholate (XLD), and SS agars (Difco) (21). In addition, 42 different pure cultures of bacteria were tested on the three agar media to verify that the soft rotting erwinias and pseudomonads would not grow or produce black or black-centered colonies at 37°C, and that *Salmonella* would produce black colonies and could be readily distinguished on SS agar. Bacteria included 12 authenticated strains of *Salmonella* (*S. sp.* serotype anatum, *S. arizonae*, *S. sp.* serotype dublin, *S. enteritidis* and *S. typhimurium*); 3 strains each of peptolytic *P. fluorescens* and *P. viridiflava*; 2 strains of *P. aeruginosa*; and a group of *Enterobacteriaceae* that included 5 strains of *Erwinia carotovora* (subsp. *carotovora*, *atroseptica*, and *wasabiae*), 3 strains of *Pantoea* (formerly *Enterobacter agglomerans*, 2 strains each of *Enterobacter aerogenes*, *E. cloaca*, *E. dissolvens*, and *E. sakazakii*, 2 strains of *Escherichia coli*, 3 strains of *Yersinia enterocolitica*, and 1 strain each of *Proteus vulgaris*, *Klebsiella pneumoniae*, and *Serratia marcescens*.

Differences in the incidence and concentration of hydrogen sulfide-positive colonies on healthy and soft rotted tissues were statistically confirmed by *t* test on Statpro statistical software (Wadsworth Professional Software, Inc., Boston, MA).

Confirmation testing of suspected strains of *Salmonella*. A collection of 166 bacteria from well-isolated black or black-centered colonies on SS agar, drawn from samples of 20 different commodities, were transferred, single-colony cloned, and maintained at 4°C on Pseudomonas Agar F (PAF, Difco), a medium we found satisfactory as a general-purpose agar for storage of bacterial cultures. Within 2 months of isolation, strains were subcultured, transferred to urea agar tubes, and incubated at 37°C for 6 to 18 h. Urease-negative strains were then grown in triple sugar iron (TSI) and lysine iron agar (LIA) slants at 37°C for 24 h. Tubes were incubated an additional 24 h if reactions were negative or borderline. Positive reactions on TSI tubes, for the H₂S⁺ strains we selected, consisted of red (alkaline) slants, yellow (acid) butts with black streaks (H₂S reactions), with or without gas production. Positive reactions on LIA consisted of purple slants and butts (alkaline) with or without H₂S within 48 h. Strains positive in the TSA/LIA slants were then tested on Roche Enterotube II (Roche Diagnostic Systems, Montclair, NJ) for the indole and Voges-Proskauer reactions, and for additional confirmation of *Salmonella* by the Enterotube II computer coding and identification system. The confirmation procedure was tested on four of the authenticated strains of *Salmonella* and on other test species as negative controls. Suspected

strains testing positive were considered presumptive *Salmonella* and then tested serologically.

Serological agglutination tests with *Salmonella* O antiserum Poly A-1 and Vi (Difco) were performed according to manufacturer's directions on strains of presumptive *Salmonella* taken from growth in TSI tubes. Those not reacting with the antiserum were tested with *Salmonella* O antiserum Poly C. Strains negative to Poly C were then tested with antiserum Poly D, and so on through antiserum Poly G. Strains reacting with any of the *Salmonella* antisera were considered positive. Strains with weak agglutination reactions were retested with the same antiserum for confirmation and then tested with the remaining antisera.

Inoculation of tissue disks with *Salmonella* and soft rot bacteria. Disks 12 mm in diameter and 3 to 4 mm thick were aseptically cut from potato, carrot, or pepper tissues. Three randomly selected disks

were placed in 60-mm petri plates and inoculated on cut surfaces with 10 µl of a bacterial suspension. Suspensions were prepared from loopfuls of 24- to 48-h cultures grown on PAF agar at 21°C, washed once, and adjusted to approximately 5 × 10⁸ CFU/ml by optical density measurements at 590 nm. Disks were inoculated with *S. typhimurium* (ATCC 14028), *E. carotovora* strain E24 (obtained from H. Moline, USDA, Beltsville, MD), or *P. viridiflava* strain 312 (obtained from C.-H. Liao, USDA, Philadelphia, PA), or with a mixture of *Salmonella* and *E. carotovora* or *Salmonella* and *P. viridiflava*. Uninoculated control disks were included. Inoculated disks were incubated at 34°C for up to 72 h and sampled at 0, 16, 24, 48, and 72 h. At each sampling, three disks per bacterial treatment were agitated in 30 ml of sterile water for 10 min, and 10 µl was serially diluted to eight logs replicated twice. Dilutions were surface-streaked on SS and on PAF agar (0.1 ml spread on 60-

Table 2. Concentration of black, hydrogen sulfide-positive colonies (suspected *Salmonella*) on *Salmonella*-Shigella (SS) agar streaked with wash water or enrichment broth from matched samples of healthy and soft rotted fruits and vegetables^a

Commodity	Paired samples	Black colonies per ml of wash water ^b		Black colonies per ml of enrichment broth ^c	
		Healthy tissues	Soft rotted tissues	Healthy tissues	Soft rotted tissues
Basil	1	0	0	0	0
Beans, snap	3	0	3.4 × 10 ⁶	5.0 × 10 ⁷	1.0 × 10 ⁸
Beet, greens	2	0	2.0 × 10 ⁵	0	1.2 × 10 ⁸
Broccoli, rabe	3	1.5 × 10 ⁴	5.0 × 10 ⁴	8.5 × 10 ⁶	1.6 × 10 ⁶
Cabbage	1	0	0	0	0
Cantaloupe	2	1.5 × 10 ⁵	5.0 × 10 ³	5.0 × 10 ⁵	4.0 × 10 ⁶
Carrot	4	1.5 × 10 ⁵	8.9 × 10 ⁶	5.0 × 10 ⁵	5.0 × 10 ⁷
Cauliflower	6	5.0 × 10 ⁴	8.3 × 10 ⁶	8.0 × 10 ⁷	4.6 × 10 ⁹
Celery	5	0	1.0 × 10 ⁵	0	1.0 × 10 ⁸
Cilantro	1	0	1.0 × 10 ⁵	0	7.5 × 10 ⁸
Cucumber	10	2.0 × 10 ⁵	8.9 × 10 ⁶	3.6 × 10 ⁹	7.8 × 10 ⁹
Dill	1	0	1.0 × 10 ⁵	0	0
Fennel	1	0	0	5.0 × 10 ⁶	0
Kale	1	0	0	0	5.0 × 10 ⁸
Lettuce, iceberg	5	0	0	0	0
Lettuce, Romaine	3	0	0	0	2.5 × 10 ⁷
Mushroom	6	0	1.7 × 10 ⁶	1.6 × 10 ⁷	1.0 × 10 ⁶
Onion, green	1	0	0	0	0
Parsley	5	0	0	0	3.7 × 10 ⁷
Pepper, bell type	13	5.0 × 10 ⁵	9.1 × 10 ⁶	2.4 × 10 ⁹	8.9 × 10 ⁹
Pepper, cubanelle	3	0	4.5 × 10 ⁵	5.0 × 10 ⁸	2.6 × 10 ⁹
Pepper, long hot	1	0	0	0	5.0 × 10 ⁷
Potato, round red	1	4.0 × 10 ⁵	5.0 × 10 ⁷	5.0 × 10 ⁷	4.5 × 10 ⁷
Potato, round white	3	5.0 × 10 ⁴	1.4 × 10 ⁷	2.5 × 10 ⁸	4.2 × 10 ⁹
Potato, russet	2	1.3 × 10 ⁵	3.8 × 10 ⁵	7.5 × 10 ⁹	1.3 × 10 ⁹
Radish	4	0	1.0 × 10 ⁵	2.5 × 10 ⁶	7.5 × 10 ⁷
Spinach	15	0	3.0 × 10 ⁵	1.7 × 10 ⁷	8.6 × 10 ⁸
Squash, yellow	2	0	2.5 × 10 ⁵	0	1.3 × 10 ⁹
Squash, zucchini	3	0	3.0 × 10 ⁵	2.3 × 10 ⁸	7.5 × 10 ⁸
Tomato	7	2.5 × 10 ⁵	1.5 × 10 ⁶	6.0 × 10 ⁷	5.0 × 10 ⁸
Tomato, plum	3	0	1.6 × 10 ⁶	0	2.5 × 10 ⁹
Watercress	2	0	5.0 × 10 ⁴	0	2.5 × 10 ⁷
Average of 120 samples		1.0 × 10 ⁵	3.7 × 10 ⁶	7.5 × 10 ⁷	2.7 × 10 ⁹
Ratio healthy:soft rot			1:37		1:37.3

^a Diseased portion of sample (10 to 60 g) separated and assayed as a paired subsample with the healthy portion (200 to 600 g).

^b Samples agitated for 30 min in 500 ml of sterile water. One ml wash diluted 10-fold, plated directly on SS agar, then incubated 24 h at 37°C.

^c One ml of wash water diluted 10-fold in selenite broth, incubated 24 h at 37°C, then plated on SS agar and incubated 24 h at 37°C.

mm plates), and incubated for 24 to 48 h at 37 and 21°C, respectively, and colonies were counted. On SS agar at 37°C, *Salmonella* appeared as black colonies after 24 h, and *Erwinia* and *Pseudomonas* did not grow. On PAF agar at 25°C, *Salmonella* could be distinguished from *Pseudomonas* by the absence of fluorescent pigments, and from *Erwinia* by colony size and texture. Calculations of viable cells, or CFU, per milliliter were based on plate counts between 30 and 300 colonies per plate (17). Four separate, replicated tests were conducted with disks cut from potato tubers, and one each from carrot and pepper.

RESULTS

Verification of methodology. Fifty-six of the specimens (28 healthy and 28 soft rotted) were tested for incidence of H₂S⁺ colonies by the modified method using only one enrichment broth (selenite cystine) and plating on only one agar (SS), and by the standard method using enrichment in selenite cystine and in tetrathionate broths and plating on HE, XLD, and SS agars. H₂S⁺ colonies appeared on 25 of the samples enriched in tetrathionate broth and on 21 of the samples enriched in selenite broth. Only 18 of the samples, however, were positive in both broths. Three of the selenite-positive samples were not positive (i.e., confirmed) in tetrathionate broth, while seven of the tetrathionate samples were not confirmed by the selenite broth. Thus, enrichment in selenite cystine broth was considered to be the more conservative procedure of the two. Positive samples

were positive on all three agars tested, the black colonies being easiest to detect on SS agar.

Of the 42 pure cultures of bacteria tested on SS agar at 37°C, all *Salmonella* strains and *Proteus vulgaris* produced black colonies. The soft rotting bacteria *Erwinia* and *Pseudomonas* did not grow or produced black colonies on SS at 37°C within the 48-h incubation period.

Prevalence and concentration of black (hydrogen sulfide-positive) colonies on fruits and vegetables. Forty-eight different types of healthy and soft rotted fruits and vegetables were sampled in the marketplace, each at least five times. Among the healthy specimens, 257 of 781 broth-enriched samples (33%) and 122 of 402 (unenriched) wash samples (30%) yielded black or black-centered colonies on SS agar (Table 1). By comparison, specimens affected with bacterial soft rot had a higher prevalence of black colonies: 316 of 533 broth-enriched samples (59%) and 264 of 401 wash samples (66%). The pattern of higher prevalence of black colonies on soft rotted than on healthy samples held for almost all commodities tested except for cantaloupe, iceberg lettuce, green onions, cherry, and plum tomatoes, in which differences were not clear. Almost all samples of alfalfa sprouts, bean sprouts, and potatoes had black colonies whether healthy or rotted. Differences between healthy and soft rotted specimens were statistically significant beyond the 99% level of confidence (paired and unpaired *t* test values of over 6) for both wash and enriched samples.

Differences between healthy and soft rotted tissues were also demonstrated with 120 pairs of samples, representing 30 different commodities, where soft rotted portions were excised as subsamples and tested against the healthy portions (Table 2). In general, counts were higher in soft rotted samples. Average number of black colonies per milliliter of wash water from the healthy tissues was 1.0×10^5 , and from soft rotted tissues was 3.7×10^6 , a ratio of 1:37. Average CFU counts per milliliter of enrichment broth were 7.5×10^7 for healthy tissues and 2.7×10^9 for soft rotted tissues, also a ratio of 1:37. Differences were statistically significant beyond the 99% level of confidence. Correcting CFU values for weight of sample, since healthy portions were generally 10 times the weight of soft rotted portions, average differences increase to 370 times. Variations occurred with cantaloupe, where wash water counts were higher for the healthy rather than the soft rotted portions, and with broccoli, fennel, mushrooms, and russet potatoes, where the same variation occurred with broth-enriched samples. In this particular series of test samples, basil, cabbage, iceberg lettuce, and green onions yielded no black colonies in either healthy or rotted subsamples.

Confirmation test for strains of presumptive *Salmonella*. Since not all black colonies on SS agar are *Salmonella*, 166 hydrogen sulfide-positive strains, plus one authentic strain of *S. typhimurium* (ATCC 14028) as a check, were tested for confirmatory physiological and serological reactions. Of the 166 strains, 110 were from soft rotted and 56 from healthy samples. Ninety-one strains were discarded as non-*Salmonella* by biochemical testing (15). Serological tests on the 75 remaining strains confirmed 50, or 30.1% of the 166, as *Salmonella*, as well as *S. typhimurium* ATCC 14028. Thirty-one of 111 strains (28%) from soft rotted samples and 19 of 55 strains (35%) from healthy samples were confirmed positive (Table 3). The 50 were also positive for *Salmonella* by the Roche Enterotube tests.

Multiplication of *Salmonella* on inoculated tissue disks. *S. typhimurium* multiplied readily at room temperature (21°C) on inoculated disks of potato, carrot, and pepper in the absence of soft rotting bacteria. From an initial average of less than 10^6 CFU/ml (from wash of three inoculated disks), populations peaked in 48 h at 4.0×10^8 CFU/ml, as determined by dilutions on PAF agar (Fig. 1A). From determinations on SS agar, *Salmonella* populations peaked at an average of 1.5×10^7 CFU/ml (Fig. 1B). No bacteria were detected from water-inoculated controls.

The presence of soft rotting bacteria significantly affected multiplication of *Salmonella*. On SS agar, 16 h after inoculation, *Salmonella* counts averaged more than 10-fold higher in disks coinoculated with *Erwinia*, and more than fivefold higher in those coinoculated with *Pseudomonas*. Similarly, as calculated from dilution plating on PAF agar, *Salmonella* counts 24 h after inoculation averaged approximately 10-fold higher when coinoculated with *Erwinia* and threefold higher with *Pseudomonas*.

DISCUSSION

Based on this study involving more than 500 samples each of healthy and soft rotted commodities collected in retail markets, the incidence of suspected *Salmonella* on produce affected by bacterial soft rot was twice that of healthy samples. Concentrations of bacteria were also affected by the presence of bacterial soft rot: there were 37 times more suspected *Salmonella* in wash from soft rotted samples than from healthy samples. Controlled experiments with tissues inoculated with one strain of *Salmonella* confirmed that bacterial soft rot infection increased multiplication of *Salmonella* by at least three- to 10-fold compared with multiplication on uninfected tissues.

The term "suspected *Salmonella*" has been used throughout to describe black or black-centered bacterial colonies appearing on SS agar incubated 24 to 72 h at 37°C. Black colonies from the wash samples

Table 3. Sources of confirmed *Salmonella* isolates

Commodity	Confirmed isolates/ isolates tested		
	Healthy tissue	Rotted tissue	Percent positive
Anise	0/1	...	0
Broccoli	...	1/2	50
Cantaloupe	0/2	...	0
Carrot	0/2	0/4	0
Cauliflower	2/2	0/1	67
Celery	3/4	1/2	67
Cilantro	1/2	...	50
Cucumber	1/1	5/11	50
Dill	...	1/1	100
Greens, beet	...	1/1	100
Lettuce ^a	2/5	0/5	20
Mushroom	0/4	1/7	9
Parsley	...	0/1	0
Pepper ^b	2/9	7/30	23
Potato	1/4	2/8	25
Radish	0/2	2/6	25
Snap beans	0/5	0/3	0
Spinach	2/4	3/5	56
Squash	1/2	0/5	14
Tomato	4/7	6/17	42
Unknown ^c	...	1/1	100
Totals	19/56	31/110	
Positive	33.9%	28.1%	30.1%

^a Iceberg and Romaine.

^b Bell type and cubanelle.

^c Records lost.

could be from a variety of H_2S^+ bacteria, including *Citrobacter*, coliforms, *Proteus*, and *Salmonella*. Since incubation in selenite cystine broth suppresses coliforms and allows small populations of *Salmonella* to grow, the likelihood of black colonies being *Salmonella* was greater in enriched samples. Nevertheless, incidence of black colonies in wash versus enriched samples were comparable, as were incidence ratios between healthy and soft rotted samples. Therefore, unenriched wash samples were a useful indication of possible *Salmonella* incidence. Confirmatory tests for a random sampling of 166 strains, 111 isolated from (enriched) soft rotted

specimens and 55 from healthy samples, and based on key physiological properties and serological reactions, showed 50 of the 166 strains (30%) to be *Salmonella*, 31 from soft rotted and 19 from healthy specimens.

In the few systematic studies in the scientific literature of incidence of *Salmonella* on fresh fruits and vegetables, percentages have generally been under 10% (12,22), with the exception of the report by Ercolani (8) in which incidences of 68 and 72% were reported for leaf lettuce and fennel, respectively. Our percentages, ranging from 30 to 33% in healthy specimens, refer to incidence of suspected *Sal-*

monella strains. Since only 30% of our suspected strains were confirmed *Salmonella*, a conservative interpretation would correct incidence values accordingly: 9 to 10% incidence in healthy samples and 18 to 20% incidence in soft rotted samples, conforming our data with those of published reports.

Bacterial soft rot on fresh fruits and vegetables, generally considered a sign of poor handling, storage, or sanitation, should also be a warning for possible enteric bacteria. While it can be assumed that *Salmonella* and other fecal coliforms that may be present in commercially handled fresh produce are at base levels, any factors that favor multiplication of bacteria before consumption could result in a public health problem. Precautions should especially be observed if there is evidence of bacterial decay in foods being prepared. Handling practices that lead to bruising and mechanical damage, the predisposing factors for bacterial soft rot, should be controlled. Finally, since *Salmonella* survive and grow on contaminated, fresh-cut surfaces of tomatoes (2,26,28) and other vegetables, sanitation and treatments such as chlorination (20,26) should be rigorously practiced in the marketing channels between the packing house and final consumption.

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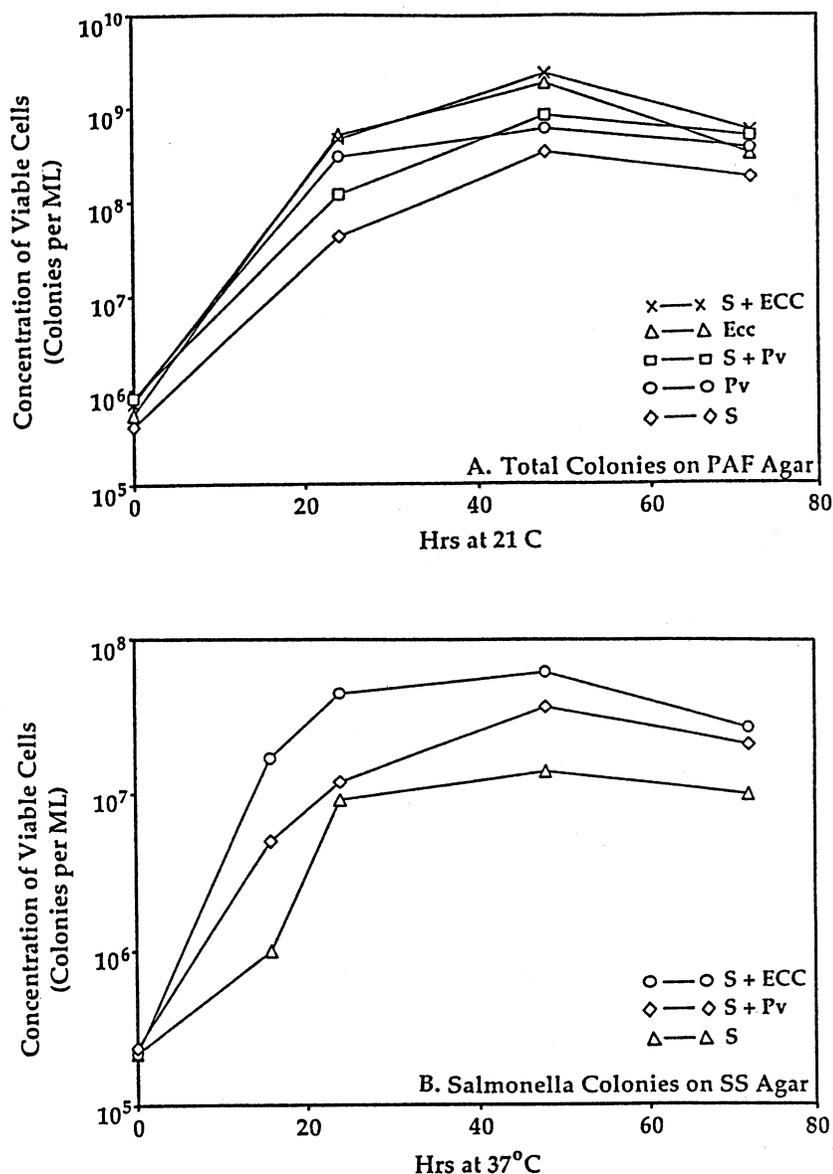


Fig. 1. Concentration of viable cells, determined as CFU/ml, washed from tissue disks inoculated with 10 μ l of bacterial suspensions (5×10^8 cells/ml), incubated at 21°C, and sampled at 0, 16, 24, 48, and 72 h. Three disks per treatment were agitated for 15 min in 30 ml of sterile water and serially diluted on two different solidified media: top, *Pseudomonas* Agar F (PAF) (incubated at 21°C for 48 h); bottom, *Salmonella-Shigella* (SS) agar (incubated at 37°C for 24 h). Dilutions were replicated twice. Inoculation treatments were with *Salmonella typhimurium* ATCC 14028 (S), *Erwinia carotovora* E24 (Ecc), *Pseudomonas viridiflava* 312 (Pv), *Salmonella* plus *E. carotovora* (S + Ecc), or *Salmonella* plus *P. viridiflava* (S + Pv). Each point represents an average of six tests, three disks per test. Potato disks were used with four of the tests, and pepper and carrot disks with one each.

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