Factors Affecting Compost Tea as a Potential Source of 
*Escherichia coli* and *Salmonella* on Fresh Produce†

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**ABSTRACT**

Compost tea (CT) is an unheated on-farm infusion of compost used as a spray or soil drench to promote plant growth and control foliar and root diseases. Because food safety involves all aspects from farm to fork, CT should meet basic microbiological criteria for water quality. This report describes the effects of two CT production processes, aerated and nonaerated, on growth and survival of foodborne pathogens and fecal coliforms. Seven commercially available nutrients used to supplement CT were tested individually and in combination for their effects on the growth of *Escherichia coli* and *Salmonella*. Compost containing 10¹ to 10³ CFU/g initial concentrations of *E. coli* O157:H7 and *Salmonella* Enteritidis were used to assess growth and survival responses to aerated CT (36-h preparations) and nonaerated CT (8.5-day preparations). Pathogen and fecal coliform populations were undetectable by 8.5 days in nonaerated CT without nutrient supplements. *E. coli* O157:H7 decreased to below detection levels in aerated CT at 36 h without the use of supplements. In contrast, the addition of commercially formulated mixtures or combinations of nutrient supplements resulted in growth of *E. coli* O157: H7, *Salmonella*, and fecal coliforms by 1 to 4 log CFU/g in both aerated and nonaerated CT. When nutrient supplements were added, aerated CT sustained higher concentrations of *E. coli* O157:H7, *Salmonella*, and fecal coliforms than did nonaerated CT. Thus, addition of supplements supports growth of human pathogens from very low initial concentrations in both aerated and nonaerated CT and should be avoided when CT is used on fresh produce.

Interest in and use of compost extracts as crop protection sprays originated with reports documenting control of plant and fruit diseases on grapes, beans, and tomatoes (3, 6, 15, 18, 27). Earlier studies with refuse compost extracts indicated that motility of sting nematodes, *Belonolaimus longicaudatus* Rau, a root pathogen, was adversely impacted by such treatments (7). Current interest from organic growers and others interested in nonchemical plant disease control has stimulated the development of a wide variety of commercially available devices and products for on-farm production of watery extracts of compost, now currently referred to as compost tea (CT). Plant protection and growth promotion efficacy data obtained with the currently available CT production devices and inputs are slowly being compiled, but results are highly variable (8, 17, 19–21). Interest in and use of CT as a spray for biocontrol of foliar and fruit diseases or as a soil drench for promotion of plant growth and/or biocontrol of root diseases (4, 8–10, 22, 25, 26) has expanded among some growers in the United States and elsewhere, particularly as more equipment and information about this on-farm practice has become available through the internet. In the United States, the National Organic Standards Board (NOSB), which advises the Secretary of Agriculture on various aspects of implementing the U.S. Department of Agriculture (USDA) National Organic Program, has recognized that in addition to the widespread use of compost there is an increasing use of CT among growers. The NOSB Compost Tea Task Force reviewed the known practical and scientific information on the subject to develop and report their recommendations (http://www.ams.usda.gov/nosb/meetings/CompostTeaTaskForceFinalReport.pdf).

In contrast to initial reports on the crop protection benefits of CT prepared without aeration or incorporation of supplements (1, 20–22), recent commercial approaches have emphasized production of aerated CT (ACT) by mechanically infusing air into the liquid during the first 18 to 36 h of brewing (8–12, 19) followed by immediate use on crops or soils. Alternatively, nonaerated CT (NCT) production methods, which involve steeping of compost in water for several days to several weeks, are still used by some to make CT on the farm. The addition of various supplements to the liquid phase at the start of the brewing period is another recent trend advocated by some producers and vendors of CT equipment (19). Some of these supplements are nutrients designed to encourage a rapid increase in the concentration and metabolic activity of bacteria and fungi extracted from the compost matrix (9, 19). Published (5) and unpublished (2, 11) data for CT and compost-water mixtures are limited, and studies have yielded conflicting results regarding the growth of *Escherichia coli* and *Salmonella* (14). Because CT is sprayed onto plant parts that could be consumed raw, it is important to understand the potential for pathogen growth in CT. When various supplements are added to the CT at the start of the brewing pro-

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cess, the growth of pathogenic microbes that may have survived composting or may be inadvertently introduced into the brewing process could be affected.

One approach to control of pathogen growth is to ensure that high quality compost with undetectable numbers of pathogens is used for CT production. The NOSB recommends that CT be prepared from compost that meets thermophilic time-temperature standards for a process to further reduce pathogens as described in the U.S. Environmental Protection Agency regulations for composting (23). Raw manure, partially composted manure, or commercial food waste are not suitable for CT production within the USDA certified organic program. Although compost containing thermophilic organisms is suitable for gardens, lawns, and soils, it cannot be considered a sterile product. Low concentrations of bacterial pathogens in a batch of thermophilic compost could increase markedly under favorable conditions, such as those possibly present in some CTs.

In this study, two CT production processes, aerated and non-aerated, were evaluated for growth and survival of foodborne pathogens and fecal coliforms. Seven commercially available CT nutrient supplements and their individual components were tested to determine their individual and combined effects on supporting growth of selected pathogens and indicator organisms.

**MATERIALS AND METHODS**

**Bacteria and culture conditions.** *E. coli* isolate 427 (serotype O157:H7; X. Jiang, Clemson University, Clemson, S.C.) originated from cattle feces and has ampicillin resistance (100 μg/ml) and green fluorescent protein traits from plasmid transformation (13). Isolate 435 (J. Meng, University of Maryland, College Park), a spontaneous mutant of *E. coli* ATCC 43895 that is resistant to nalidixic acid (50 μg/ml), was isolated from ground beef implicated in an outbreak of foodborne disease. *E. coli* isolate 466 was isolated from dairy manure compost at the USDA facility (Beltsville, Md.), *Salmonella Enteritidis* isolate 430 (X. Jiang) is a spontaneous mutant that is resistant to ampicillin (100 μg/ml). *Salmonella Senftenberg* isolate 695 (J. Meng) is a spontaneous mutant that is resistant to nalidixic acid (50 μg/ml). *Enterococcus faecalis* isolate 476 was isolated from poultry manure–based compost at the USDA facility. The identities of all bacteria used in this study were confirmed using standard microbiological methods, including biochemical and serological methods (24). Cultures were stored at −80°C in Trypticase soy broth (TSB; Difco, Becton Dickinson, Sparks, Md.) containing 30% glycerol. *E. coli* isolates were grown and enumerated on MacConkey agar (Difco, Becton Dickinson) supplemented with 100 μg/ml ampicillin (Sigma Chemical Co., St. Louis, Mo.) or 50 μg/ml nalidixic acid (Sigma). *Salmonella* isolates were grown and enumerated on XLT4 agar (Difco, Becton Dickinson) supplemented with 50 μg/ml nalidixic acid (Sigma). The *Enterococcus* isolate was grown and enumerated on modified *Enterococcus* agar (Difco, Becton Dickinson) supplemented with 1.5% agar (Difco, Becton Dickinson).

**Compost source and nutrient supplements.** Compost used throughout the study was commercially available and marketed specifically for CT production (Rexius, Eugene, Oreg.) and is a proprietary blend of several thermophilic composts produced from animal manure and yard trimmings. Compost was collected from a mature pile at the production facility and stored indoors at ambient temperature until used.

The three nutrient treatments were selected because they represent approaches currently used by some growers producing CT on their farms. Treatment A was the basic CT without addition of commercially available supplements. The only nutrients available in this treatment were those naturally present in the 500 g of compost placed in the brewing assembly filter sock. Treatment B was identical to treatment A except that 0.5% (vol/vol) Bacterial Nutrient Solution (Soil Soup, Inc., Seattle, Wash.) was added at the start of the brewing cycle to enhance bacterial growth purported to be beneficial in controlling foliar phytopathogens (as stated by users and the manufacturer). This bacterial nutrient solution is a proprietary blend of molasses, bat guano, sea bird guano, soluble kelp, citric acid, Epsom salts, ancient seabed minerals, and calcium carbonate. Treatment C was identical to treatment A except that the following supplements were added in accord with recent recommendations by CT users and vendors: 0.12% (wt/vol) powdered soluble kelp (Maxicrop, Elk Grove Village, Ill.), 0.25% (wt/vol) liquid humic acids (Humax, JH Biotech, Inc., Ventura, Calif.), and 0.96% (vol/vol) glacial rock dust (Gaia Green Products Ltd., Grand Forks, British Columbia, Canada) per 15 liters to encourage growth of microbes purported to aid in biocontrol of phytopathogens.

**CT studies.** Compost tea was prepared with aeration (ACT) and without aeration (NCT) using a brewing time within the range typical for each process: 36 h for ACT and 8.5 days for NCT. Each ACT batch was made with a Bio-blender (Soil Soup), which is immersed in and actively infuses air into the CT throughout the brewing process. Tap water (15 liters per bucket) was aerated for 2 h to dechlorinate the water prior to use. Compost with inocula was added to the liquid by completely immersing the filter sock containing the compost into the water, which contained the designated nutrient supplements (described above) according to the treatment. To assist extraction of soluble materials and microorganisms from the compost, the filter sock was lifted above the water and allowed to drain into the bucket for 15 s and then reimmersed for 30 s. This action was performed three times, and the filter sock was left in the liquid during aeration for the remainder of the production cycle.

The NCT process is the simplest approach for on-farm preparation of CT without aeration because no commercial apparatus is necessary. Each NCT batch was made by allowing 15 liters of tap water (20 to 22°C) to passively dechlorinate before the compost (500 g) was mixed directly with the water. Compost socks are typically not used for NCT production to facilitate microbial extraction, but the final product is strained prior to spraying. Nutrient supplements were then added as designated for each of the treatments. The entire batch was vigorously stirred for 20 s with a sterile glass rod and then left undisturbed for 204 h. Each complete set of CTs was prepared once each month for 3 months, constituting three replications over time.

Bacterial isolates were grown individually overnight in 250-ml Erlenmeyer flasks containing 150 ml of TS broth at 37°C, centrifuged at 2,683 × g, washed three times, and then resuspended in 100 ml of sterile phosphate-buffered saline, pH 7.4 (PBS). For the CT component bioassay, each isolate (USDA isolates 435 and 695) was serially diluted in sterile PBS and enumerated on MacConkey agar. All cultures were maintained on wet ice until inoculated into each nutrient source (maximum 18 h).

For the ACT-NCT study, pathogen inocula (USDA isolates 435, 427, 466, 430, and 476) were conditioned to grow on the compost substrate to avoid abrupt nutritional and consequent met-
abolic shifts that can accompany growth when changing from synthetic nutrient media to complex natural compost nutrients. By conditioning the inocula on the compost substrate prior to introduction of a small aliquot into the mixed microflora of a larger mass of compost, the inoculum simulates as closely as possible natural contamination by a small number of bacteria that could survive in an inadequately heated portion of compost. The conditioning involved steam pasteurizing a portion of compost for 1 h on each of three consecutive days. This conditioned compost was then dried at 80°C for 24 h before inoculation. No detectable microbes were found in this pasteurized compost before inoculation. Steaming avoided the potential production of reduced forms of iron, manganese, and other elements that can develop and impart toxicity to the substrate when composts or soils are autoclaved (28). Cell suspensions of each isolate (100 ml, prepared as above) were inoculated in individual 100-g portions of the oven-dried, steam-pasteurized compost to achieve 50% moisture content (wt/vol). Inoculated compost was incubated for 72 h at 22°C to allow isolates to adapt to growth (without competition) within the pasteurized compost matrix, and then the bacterial populations were enumerated. Inoculated compost was stored at 4°C until used. When used for CT production tests, inoculated compost was diluted 10-fold with stored uninoculated, unsteamed compost as received from the supplier to achieve the target starting concentrations (approximately 10^4, 10^5, and 10^6 CFU/g) for CT brewing. For example, the inoculated compost (10 g) was added to 90 g of uninoculated compost in sealed plastic bags and homogenized by vigorously shaking and manually massaging for 5 min. After it was determined that the target concentrations of pathogens were present, a small portion (approximately 5 g) of the pathogen-containing compost was placed in the center of a larger mass (approximately 495 g) of uninoculated, unsteamed, as-received compost, and the entire 500 g was loaded into autoclaved 100-ml mesh filter socks marketed for CT production (Soil Soup). Filter socks were placed into sterile plastic bags, sealed, and shipped overnight in secure insulated coolers with frozen gel packs to Corvallis, Oreg., for subsequent brewing.

All buckets containing human pathogens were brewed in an ambient temperature growth chamber to simulate on-farm conditions. Samples (50 ml) were aseptically drawn (36 h for ACT and 8.5 days for NCT) from the center of each bucket 10 cm below the surface of the liquid. Samples were stored at 4°C overnight prior to analysis.

Component bioassays. For the CT component bioassays, seven different materials currently marketed as possible alternatives to molasses in CT production were evaluated for their ability to support growth of E. coli O157:H7 isolate 435 and Salmonella Senftenberg isolate 695. Components were diluted 0.5% (vol/vol) in sterile deionized water and inoculated with either E. coli at 1.35 log CFU/ml or Salmonella at 1.42 log CFU/ml. After 24 h of incubation at 22°C, microbial populations were enumerated, and each bioassay was performed in triplicate.

Microbiological and physical characterization of compost and CTs. Samples of compost and CT were serially diluted in buffered peptone water (Becton Dickinson) and plated in duplicate (50 μl on each plate) using a WASP-II spiral plating instrument (Don Whitley Scientific, Ltd., Shipley, UK) to enumerate bacterial populations. The detection limit for this procedure was calculated as 10 CFU/ml. Inoculated isolates of E. coli were enumerated on MacConkey agar containing antibiotics, incubated for 24 h at 37°C, and appeared as pink colonies. Fecal coliforms were enumerated as pink colonies on MacConkey agar incubated at 44.5°C for 18 to 24 h. Inoculated Salmonella colonies were enumerated as black colonies on XLT4 agar containing antibiotics that was incubated at 37°C for 24 h.

E. coli spp. were enumerated on modified Enterococcus agar (Difco, Becton Dickinson, adjusted to 1.5% agar concentration) incubated at 37°C for 48 h and appeared as red or purple colonies. Indigenous baseline E. coli and Salmonella concentrations were determined on the appropriate media by streaking CT prepared using uninoculated and unsteamed compost.

At the final sampling event (36 h for ACT and 204 h for NCT), temperature, pH (model 150 pH meter, IQ Scientific Instruments, San Diego, Calif.), electrical conductivity (model 933100, Hanna Instruments, Woonsocket, R.I.), and dissolved oxygen (model 600, Engineered Systems and Design, Newark, Del.) were recorded for each bucket. Moisture content of compost samples was determined by oven drying approximately 50-g samples at 105°C overnight. Results were used to calculate dry weight for reporting bacterial concentration in the compost.

Statistical analyses. Analyses of variance (ANOVAs) with individual t tests were performed to determine the significance of differences between population means in response to different nutrient, supplement, and growth conditions. Comparisons of mean log values for each strain were made using the least significant difference separation technique of the mixed ANOVA in the SAS/STAT software (version 9.13, SAS Institute Inc., Cary, N.C.).

RESULTS

CT studies. Comparative analysis of all inoculated pathogen populations across all treatments revealed a very strong significant growth response in treatments B (bacterial nutrient supplement, P < 0.0001) and C (kelp, humic, and rock dust supplement, P < 0.0001). Analysis of individual isolates revealed that populations of E. coli O157: H7 435 and 427, Enterococcus 476 (both ACT and NCT), and Salmonella Enteritidis 430 (NCT only) were reduced below inoculum concentrations in response to treatment A (Figs. 1 and 2). In the 36-h ACT from treatment A, Salmonella and fecal coliform concentrations increased approximately fivefold from the inoculum concentrations (Fig. 1). In contrast, the concentrations of pooled populations of E. coli O157:H7 and fecal coliforms (ACT and NCT) in treatments B (P < 0.0065) and C (P < 0.0001) were significantly higher than those of the inoculum (Figs. 1 and 2). In general, the populations of E. coli O157:H7, Salmonella Enteritidis, and Enterococcus 476 were lower in the NCT than in the ACT production systems.

In 8.5-day NCT (Fig. 2), both Salmonella and Enterococcus were undetectable in treatments A and B. Salmonella populations increased 10-fold (from inoculum concentrations) in response to treatment C. E. coli O157:H7 populations also increased but to a greater extent (1,000-fold) with treatment C than with treatment B. Fecal coliforms survived and grew more than 1,000-fold in response to both treatments B and C, but they were undetectable in treatment A. Across all NCT treatments, the mean dissolved oxygen concentration was 2.6 ppm, the pH was 5.7, and the electrical conductivity was 0.87 (21).

For 36-h ACT (Fig. 1), in comparison with inoculated levels, Salmonella populations increased 100-fold (from inoculum concentrations) in response to treatment B and 10-fold in response to treatment C (Fig. 1). However, Salmo-
FIGURE 1. Foodborne pathogen survival in aerated compost tea (ACT, 36 h) prepared using three treatments: A, no added nutrients; B, bacterial nutrient solution (0.5%, vol/vol); C, soluble kelp (0.12%, wt/vol), humic acid (0.25%, vol/vol), and rock dust (0.96%, wt/vol). Asterisks indicate significant difference from the inoculum concentration for each species. * P < 0.05, ** P < 0.01, *** P < 0.001.

**nella** grew only slightly (approximately fivefold) in response to treatment A. *E. coli* O157:H7 isolates were undetectable in response to treatment A. *E. coli* O157:H7 and fecal coliform populations increased 100- to 1,000-fold in response to treatments B and C (Fig. 1). *Enterococcus* concentrations decreased approximately fivefold in response to treatment C but increased 10-fold in response to treatment B. Across all ACT treatments, the mean dissolved oxygen concentration was 8.25 ppm, the pH was 6.9, and electrical conductivity was 3.52 (21).

No indigenous strains of fecal coliforms, *E. coli*, or *Salmonella* were recovered on the test media streaked with CT produced from unsteamed, uninoculated, as-received compost. Across all treatments for NCT and ACT, the total heterotrophic aerobic bacterial population was significantly greater (*P* < 0.0057) in 36-h ACT (approximately 7.6 log CFU/ml) than in 8.5-day NCT (approximately 6.7 log CFU/ml). In the ACT, treatments B and C produced comparable but significantly higher concentrations of heterotrophs than did treatment A (*P* < 0.05). In the NCT, treatment C produced significantly higher concentrations of heterotrophs than did treatment B (*P* < 0.05), but concentrations in treatments B and C were not different from those in treatment A.

**Component bioassays.** Analysis of individual isolates in the component bioassays revealed that the inoculated *Salmonella* and *E. coli* grew (Table 1) even in treatments supplemented with materials that are not considered to be nutrient sources, such as humic acid.

**DISCUSSION**

The addition of certain supplements during the ACT and NCT brewing cycles (treatments B and C) contributed to the increased populations of *E. coli* O157:H7, *Salmonella* Enteritidis, *Enterococcus*, and fecal coliforms in the final CT product. Thus, reduced use of supplements in CT production is highly likely to reduce the growth of foodborne pathogens. *E. coli* O157:H7 was not detectable in all ACT and NCT where nutrient supplements were not added (treatment A). In general, where nutrient supplements were used (treatments B and C), the concentrations of fecal coliforms, *E. coli* O157:H7, *Enterococcus*, and *Salmonella* Enteritidis were always greater for the ACT than for the NCT. In contrast, Kannangara et al. (14) reported increased concentrations of *E. coli* K-12 in their NCT as compared with their ACT systems with molasses and kelp supplements. However, their results with *E. coli* K-12 in ACT agree with our results with *E. coli* O157:H7, i.e., no growth in CT without supplements. Furthermore, the *E. coli* K-12 inoculum concentrations that Kannangara et al. (14) used, 10⁶ to 10⁷ CFU/ml, are unlikely to occur in thermophilically composted manure.

Results from this study revealed that compost extracts alone may contain sufficient nutrients (treatment A) to sup-
port survival and/or growth of *Salmonella*, fecal coliforms, and *Enterococcus* spp. in 36-h ACT. However, the NCT without nutrient supplement (treatment A) contained no detectable fecal coliforms, *Enterococcus* spp., *E. coli* O157: H7, or *Salmonella* Enteritidis.

The efficacy of CT for phytopathogen control and promotion of plant growth is still unclear, and the mechanisms by which various diseases are reduced likely vary (20). One possible explanation for the effect of CT is that the phylloplane microflora is overwhelmed by the increased number of microbes from the CT spray that competitively exclude and/or inhibit the phytopathogens. Another theory is that physical barriers are created from the microbial biofilm that forms when CT is sprayed onto the foliage. However, as is the case for any water source used for crop irrigation and spray, the presence of human pathogens is a potential hazard for consumers of fresh produce. Foodborne pathogens in biofilms may be protected against environmental stressors and microbial competitors (16). If human pathogens are present even in small concentrations in compost used to prepare CT and if conditions of CT production allow these pathogens to grow, then the likelihood of contaminants being retained on foliage increases substantially when the CT is used as a spray. Therefore, control of foodborne pathogen growth during production of CT is essential for reducing the potential hazard from on-farm introduction of these pathogens through CT application. Quality control and assurances for the compost and the composting and CT production processes would help ensure against undesirable bacterial contaminants in sprays and crop and soil drenches used on fresh produce crops.

The effect of nutrient supplementation on the efficacy of phytopathogen biocontrol by CT remains a topic for fur-
ther research. Presently, no published peer-reviewed reports have substantiated reliable benefits of nutrient supplementation for increasing the efficacy of biocontrol (19). However, several reports (3–5, 12, 18–21) have documented biocontrol benefits from watery extracts of compost in the absence of nutrient supplements. Results reported here for survival and growth of foodborne pathogens in ACT and NCT correspond with those of other studies of plant growth and protection responses (21). Nutrient supplementation increases the general heterotrophic bacterial populations in CT (8–10, 12); however, there is no unequivocal evidence that the addition of supplements provides significantly better and more reliable plant protection, biocontrol, or plant growth (15, 21). Although the practice of amending CT with nutrients or supplements has expanded and engaged many produce growers in the organic production sector, our results further support the limited existing evidence (5) of the potential hazards associated with this practice. Amendment of CT with supplements increases the likelihood that populations of bacteria associated with foodborne illness will increase during the brewing process. This increased potential for pathogen growth is present even when very low concentrations (<2.0 CFU/g) of E. coli O157:H7 and Salmonella are present in the starting compost used to produce the CT. Several commercially available CT supplements (e.g., humic acid and kelp) in addition to those reported by Duffy et al. (5) support growth of E. coli O157: H7 and Salmonella during CT production despite the fact that they purportedly are added for their mineral and physical attributes rather than their carbonaceous nutrient constituents. Results suggest that these commercially available supplements contain growth supporting nutrients.

Another adverse effect observed in treatments B and C, in which supplements were incorporated into the initial liquid phase, was the development of a substantial, easily visible biofilm on all the brewing equipment, including the bucket, filter sock, and aeration device. Microbial biofilms are known to protect microbes against the deleterious effects of disinfectants, mechanical washing, and antibiotics (16). The equipment used in this study was thoroughly sterilized before CT production. It is essential that on-farm producers be educated about the necessity of sanitizing their CT equipment to prevent microbial cross-contamination between batch preparations, particularly where supplements are being used. Bacterial biofilms generated in the CT vessel or on the aeration equipment can contaminate the next batch of CT if the equipment is not properly sanitized. If such biofilms contain pathogenic microbes, such a scenario would enhance the likelihood of introducing pathogens associated with foodborne illness into a crop production system.

When CT is produced with compost or supplements that are known to support the growth of E. coli O157:H7 or Salmonella, the resulting CT should be tested for the presence of such pathogens before it is used for spraying or soil drenching of fresh produce. This recommendation is consistent with other recommendations made by the Compost Tea Task Force in its 13 October 2004 report to the NOSB.

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