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Fate of manure-borne pathogen surrogates in static composting piles of chicken litter and peanut hulls

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ABSTRACT

The fate of manure-borne pathogen surrogates (gfp-labeled *Escherichia coli* O157:H7 and *Listeria innocua* and avirulent *Salmonella* Typhimurium) in the field was monitored at both sub-surface (30 cm from surface) and surface sites of static composting piles (3.5-m base diameter) composed of chicken litter and peanut hulls. Despite exposure to elevated temperatures, *Salmonella* was detected by enrichment culture in sub-surface samples following 14 days of composting. In surface samples, pathogen surrogates were detected in the summer after 4 days of composting by enrichment culture only, whereas *E. coli* O157:H7 and *L. innocua* remained detectable by direct plating (>2log$_{10}$ cfu/g) up to 28 days in piles composted during the fall and winter. All three types of bacteria remained detectable by enrichment culture in surface samples composted for 56 days during the winter.

1. Introduction

Livestock can harbor a number of human pathogens in their gastrointestinal tract and consequently animal manure serves as a potential vehicle for zoonotic pathogens, including *Escherichia coli* O157:H7, *Salmonella* spp. and *Listeria monocytogenes* (Zhao et al., 1995; Pell, 1997). Once excreted from the animal, pathogen survival is dependent on the storage conditions. For example, Williams and Benson (1978) determined that *Salmonella enterica* serovar Typhimurium survived for at least 18 months in chicken litter at 11 or 25 °C, and 13 days at 38 °C. Likewise, decimal reduction times for *S.* Typhimurium and *E. coli* O157:H7 in poultry manure were affected by storage temperature; however, inactivation was slower in manure slurries than piles (Himathongkham et al., 2000). Nicholson et al. (2005) also reported reduced inactivation of pathogens in livestock slurries compared to manure heaps and attributed this disparity to elevation of temperatures within the solid mass. Incorporation of a carbon amendment is recommended to facilitate the metabolic activity of thermophilic microorganisms during composting to ensure that pathogens in manures are exposed to elevated temperatures and inactivated quickly.

Two major types of composting operations are used, i.e., static piles and bins, and windrows (long rows of compost turned on a regular basis). US Environmental Protection Agency's time–temperature guidelines for composting operations specify that a minimum temperature of 55 °C be maintained for a period of three consecutive days for static piles, whereas for windrows, temperatures greater than 55 °C should be maintained for at least 15 days with a minimum of five turnings during the high temperature period (US EPA, 1999). The extended period in the latter guideline is indicated to ensure that all material is exposed to the high-temperature core of the windrow for at least three consecutive days. Despite the large body of work that supports these guidelines, there are a number of studies that have found that even when the specified conditions are achieved, one or more pathogens remained viable for all types of composting systems (Wichuk and McCartney, 2007). For example, Grewal et al. (2007) observed the extended survival of low numbers of both *Listeria* and *Salmonella* inoculated into swine manure amended with sawdust and held at 55 °C. Moreover, evidence that survival occurs in commercial practices was determined by Hay (1996) who surveyed 72 composting facilities in the United States and found that more than half of the facilities monitoring for *Salmonella* spp. produced products still contaminated with the pathogen despite meeting the time–temperature criteria. Explanations for the discrepancy between expected and actual pathogen reductions include: temperature monitoring sites do not accurately reflect the temperatures encountered by the entire composting mass (Gerba et al., 1995); and recontamination of interior portions from contaminated surface sites occurs during turning of the material (Pereira-Neto et al., 1986).

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Temperature variation within a compost pile is to be expected, with heat loss proportional to the surface area of the compost mass and heat generation proportional to the volume of the compost mass. Hence, substantial temperature variations have been observed in all types of full-scale composting systems in all weather conditions. For example, in a mixed refuse and sludge aerated static pile, Pereira-Neto et al. (1987) observed a peak difference of more than 40 °C between the highest and lowest temperatures at a single point in time. Similarly, although not as dramatic, Insam et al. (1996) determined that the outer zone of unturned manure heaps remained approximately 20 °C cooler than the heap core. Correlated to these disparities in temperature were inactivation rates of *Salmonella* Senftenberg W775 that were more than five times less in the outer zone of sewage sludge composting piles compared to the center and top parts (Paluszak et al., 2003). Shepherd et al. (2007) reported that *E. coli* O157:H7 survived at the heap's surface of dairy manure compost piles for up to four months, whereas the pathogen survived for 5 and 14 days at internal locations when inoculated at initial concentrations of 10^5 and 10^7 cfu/g, respectively. In contrast, Hutchison et al. (2005) were unable to isolate *E. coli* O157:H7, *Salmonella*, or *Listeria* in surface samples of livestock waste and bedding heaps composted for 8 days and attributed their negative results to the additional contribution of sunlight exposure. Given that surface sites may be the limiting factor for producing pathogen-free composts, this study explored the fate of three pathogen surrogates (Shiga toxin-negative *E. coli* O157:H7, *Listeria innocua*, and avirulent *S.* Typhimurium) at the surface and near-surface sites of static composting piles of chicken litter and peanut hulls. In addition, indigenous populations of *E. coli* were monitored to determine their suitability as an indicator of pathogen inactivation.

2. Methods

2.1. Strains

Three avirulent bacteria were used in this field study as pathogen surrogates and included an avirulent strain of *S. enterica* serovar Typhimurium (χ3985 Δcrp-11 Δcya-12) obtained from Roy Curtiss III (Washington University, St. Louis, MO), a nontoxigenic *E. coli* O157:H7 strain B6914 obtained from J.S. Karns and J.V. Gagliardi (US Department of Agriculture, Agricultural Research Service [USDA-ARS], Beltsville, MD), and a strain of *L. innocua*. The latter two organisms were modified with a green-fluorescent protein and an antibiotic-resistant marker to enable the isolation and differentiation of colonies from the complex microflora of compost (Sambrook et al., 1989).

2.2. Inoculum preparation

Pathogen cells (*S.* Typhimurium, *E. coli* O157:H7, and *L. innocua*) were thawed from a frozen stock culture and streaked onto tryptic soy agar (TSA; Neogen, Lansing, Mich.), TSA containing 100 μg/ml ampicillin (TSA-A), and brain heart infusion agar (Becton Dickson, Sparks, MD) containing 8 μg/ml erythromycin (BHIA-E), respectively. Plates were incubated for 20–24 h at 37 °C. A single colony was selected from each of these plates and streaked onto a second plate before incubating for another 20–24 h at 37 °C. A single colony of *S.* Typhimurium, *E. coli* O157:H7, or *L. innocua* was transferred into tryptic soy broth (TSB; Neogen), TSB containing 100 μg/ml ampicillin (TSB-A), or brain heart infusion broth (Becton Dickson) containing 8 μg/ml erythromycin (BHIB-E), respectively, before incubating at 37 °C for 20–24 h with agitation (150 rpm). Cells were recovered from the broth by centrifugation (4050*g*, 15 min, 4 °C) and cell pellets were washed three times with 0.1% peptone water (Becton Dickson). The individual strains were each reconstituted with 0.1% peptone water to an optical density of 0.5 at 630 nm (approximately 10^9 cfu/ml). Cell populations of *S.* Typhimurium, *E. coli* O157:H7, and *L. innocua* were determined by plating on TSA, TSA-A, and modified Oxford media (Acumedia Manufacturers, Lansing, Mich.) containing 10 mg/ml buffered colistin methane sulfonate, 20 mg/ml buffered moxalactam solution, and 8 μg/ml erythromycin (MOX-E), respectively.

2.3. Compost feedstocks and static pile formation

Chicken litter and one year-old peanut hulls were obtained from local sources near Statesboro, Georgia. The materials were mixed thoroughly with a front-end loader and distributed into piles having a base diameter of ~3.5 m, height of ~1.2 m, and initial carbon:nitrogen (C:N) ratios from 12 to 24.

2.4. Experimental design

Six static compost piles were formed and sampled at three different times during the year: summer (July 24–August 10), fall (September 25–October 23), and late fall/early winter (November 13–January 8). Following formation, three portions (~3 kg each) were removed from each pile and placed in sterile buckets. A concentrated culture of each pathogen surrogate was sprayed on separate portions of the compost mixture and mixed thoroughly with a sterile spoon to yield 4–8$\log_{10}$ cfu/g. Inoculated samples were then portioned (~100 g) into Tyvek pouches (DuPont, Wilmington, DE.) having gas-, water- and light-permeable properties that would provide conditions within the bag similar to those encountered by microbes exterior to the bag. Twelve packages from each pathogen surrogate-inoculated mixture were left open and positioned equidistantly in each pile on the surface (30 cm from the ground), and another twelve packages were sealed, attached to a colored string, ~1 m in length, and positioned equidistantly in each pile at sub-surface locations (30 cm from both the surface and ground) with the string hanging out of the pile for pathogen identification and retrieval. Two packages from each of the pathogen surrogate-inoculated compost mixtures were removed from each location in each of the piles at each of 6 sampling times. Based on anticipated differences in temperature-related survival, sampling times varied with the location and season in which the piles were formed, spanning 14, 28, and 56 days for surface locations in summer, fall, and late fall/early winter trials, respectively, and 14, 28, and 28 days for sub-surface locations in summer, fall, and late fall/early winter trials, respectively. Concomitant with removal of packages, temperature and oxygen readings were obtained with a Demista OT-21 oxygen probe (Arlington Heights, IL) on each compost pile at surface and sub-surface locations. Surface packages were sealed and together with sub-surface packages were sent by overnight express to the Center for Food Safety's microbiological laboratory where they were analyzed for pathogen surrogate populations, indigenous *E. coli* cell numbers, pH, and moisture.

2.5. Microbiological analyses

Enumeration of pathogen surrogates was determined by direct plating, but when colonies were not detected by this method, selective enrichment was used to assay for the presence of pathogen surrogates. For direct plating, duplicate compost samples (5 g) were added to 45 ml of 0.1% peptone water in a sterile Whirl-Pak bag and mixed in a stomacher. Serial dilutions of these homogenates were spread on Petrifilm™/coliform plates (3 M, St. Paul, Minn.) for enumeration of indigenous *E. coli* populations. Serial