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Effect of heat-shock treatment on the survival of *Escherichia coli* O157:H7 and *Salmonella enterica* Typhimurium in dairy manure co-composted with vegetable wastes under field conditions

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ABSTRACT

This study investigated the survival of heat-shocked (HS) and non-heat-shocked (NHS) *Escherichia coli* O157:H7 and *Salmonella enterica* Typhimurium when co-composting dairy manure and vegetable wastes in a field setting. In the summer, HS *E. coli* O157:H7 and *Salmonella* survived for 7 and 2 days longer at the surface and bottom locations of the compost heaps, respectively, than NHS cultures. Both HS and NHS *E. coli* O157:H7 and *Salmonella* were detectable in all compost samples for more than 60 days in the winter. The results indicate that composting dairy manure with vegetable wastes under sub-optimal conditions may allow extended survival of pathogens in the heap at low ambient temperature. Analysis of covariance revealed that the heat-shock treatment may have induced cross-resistance to desiccation, allowing extended survival of HS *E. coli* O157:H7 and *Salmonella* at the surface of the compost heaps during the summer.

1. Introduction

Livestock wastes, e.g., cattle manure, may contain human pathogens, such as *Escherichia coli* O157:H7 and *Salmonella* spp. These pathogens are two of the most common causes of bacterial foodborne illnesses in the United States (Mead et al., 1999). As these microorganisms are disseminated through the fecal–oral route of transmission, most illnesses associated with these bacteria are linked to either direct or indirect contamination of food with animal wastes. This is problematic in the agricultural industry, especially regarding fresh produce production. In recent years, *E. coli* O157:H7 has been linked to the contamination of lettuce (MDH, 2005) and spinach (FDA, 2006), whereas tomatoes (CDC, 2007) and peppers (CDC, 2008) have been contaminated with *Salmonella* spp. Importantly, it was determined that animal wastes were a possible source of the *E. coli* O157:H7 strain implicated in the spinach outbreak of 2006 (CFERT, 2007).

Composting animal wastes can inactivate pathogens while creating an amendment beneficial for application to arable agricultural land. Studies have reported that the application of animal waste-based composts has improved physical conditions of soil (Leroy et al., 2008) and the growth of tomato seedlings (Ribeiro et al., 2007), and increased yields of agricultural commodities such as maize grain (Smiciklas et al., 2008). Though composting has been widely used as a way to create a valuable product from animal wastes, it can also effectively recycle plant-based wastes. It is estimated that 18.9 billion pounds of fresh fruits and vegetables are discarded by food service and retail establishments, and consumers annually (Kantor et al., 1997). Although vegetable wastes can be used as a compost constituent and may be a good nutrient source for microorganisms during composting, there is a lack of information on pathogen survival when animal wastes are co-composted with vegetable wastes.

Guidelines for carbon:nitrogen (C:N) ratios and moisture contents of composting materials have been described (Sherman, 1999). Thus, performing composting outside of the suggested guidelines may allow for the extended survival of pathogens through heat adaptation due to exposure to sublethal temperatures. The heat-shock response, induced by the activation of the *rpoS* gene, a major regulator in bacterial stress responses (Loewen and Hengge-Aronis, 1994), may have a significant effect on bacterial survival. Heat-adapted pathogens may survive for extended period of time within the body of the compost heap, possibly causing regrowth within the heap during the curing phase of the composting process. The goal of this study was to determine how heat-shocked (HS) and non-heat-shocked (NHS) cultures of *E. coli* O157:H7 and *Salmonella* Typhimurium survived in cattle manure co-composted with vegetable waste under optimal and sub-optimal conditions in a field setting.

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2. Methods

2.1. Compost heap construction

Two composting trials were conducted, with the first one in the summer (July–August, 2007) and the second one in the winter (January–March, 2008). In the months of composting in the summer, the average high and low temperatures range from 32 to 33 °C and 19 to 20 °C, respectively. In the winter months in which the second trial was performed, the average high and low temperatures range from 11 to 18 °C and −1 to 4 °C, respectively. In each composting trial, a sawdust-dairy manure mix, wasted feed, old hay and vegetable wastes were composted. In the summer, the vegetable wastes were squash lettuce and residues of their plants, whereas beans, radishes and their plant residues were used in the winter trial. The vegetable wastes were cut with small machetes into small pieces (ca. 2–5 cm), and the materials used for composting were thoroughly mixed in appropriate ratios (Table 1) then split into two heaps of equal size with the aid of a front-end loader. The heaps were constructed to a size (ca. 1.2 m in height × 2 m in width) as described by Shepherd et al. (2007), which would retain heat and allow thermophilic composting to occur. The heaps were maintained on a 25 by 16 m concrete slab which was enclosed with a gated fence. The heaps in each trial were exposed to all conditions of the natural environment.

2.2. Bacterial culture preparation

Avirulent green fluorescent protein (GFP)-labeled *E. coli* O157:H7 B6914, provided by Dr. Pina Fratamico [US Department of Agriculture (USDA), Agricultural Research Service, Eastern Regional Research Center, Wyndmoor, PA] and *Salmonella enterica* Typhimurium 8243, provided by Dr. Roy Curtiss III (Washington University, St. Louis, MO), induced to rifampin resistance (Rif^r) at 100 μg/ml, were used. A frozen stock of GFP-labeled *E. coli* O157:H7 was streaked on tryptic soy agar (TSA) (Becton Dickinson, Sparks, MD) with 100 μg/ml of ampicillin (Sigma Chemical Co., St. Louis, MO) (TSA-A), whereas Rif^r *Salmonella* was streaked on TSA containing 100 μg/ml of rifampin (Fisher Scientific, Fair Lawn, NJ) (TSA-R). The cultures were prepared as described previously (Shepherd et al., 2007).

To produce heat-shocked (HS) cultures, the time and temperature regimen employed by Mackey and Derrick (1987) was used. One flask of each overnight culture was exposed to heat treatment at 48 °C for 30 min in a circulating water bath (Haake V26; Thermo Fisher Scientific Inc., Waltham, MA). Non-heat-shocked (NHS) culture flasks were held at room temperature for 30 min. After the heat-shock treatment, the cultures were sedimented by centrifugation at 5000×*g* for 10 min, washed twice, and then resuspended in sterile 0.85% saline.

Table 1
Initial parameters, biotic and abiotic analyses of material used for composting.

Parameter	Description or results	
	Summer trial	Winter trial
Compost heap dimensions	Conical shape, ca. 2 m in base width and ca. 1.2 m in height	
Compost mixture	Cow manure – calf barn sawdust bedding, feed waste, old hay, vegetable waste[a]	
Compost mixture ratio	10:2:2:2	15:3:3:3
C:N ratio	22.4:1	17.8:1
pH	8.35	8.20
Moisture content	45.1%	56.5%
Mesophilic bacterial counts	1.6×10^8 CFU/g	2.4×10^8 CFU/g
Thermophilic bacterial counts	9.3×10^7 CFU/g	6.5×10^6 CFU/g
E. coli O157:H7	Absent in 25 g	Absent in 25 g
Salmonella spp.	Absent in 25 g	Absent in 25 g

[a] Vegetable wastes used in both trials are described in the Section 2.

2.3. Compost sample preparation

Resuspended NHS and HS cultures of *E. coli* O157:H7 and *Salmonella* cultures were inoculated into finished dairy-manure-based compost (ca. 60% moisture content) at a ratio of 1:100 (volume:weight) with the use of a spray nozzle sterilized with 70% ethanol and rinsed with sterile saline. Both HS *E. coli* O157:H7 and *Salmonella* cultures were inoculated into the same finished compost portion, whereas the NHS *E. coli* O157:H7 and *Salmonella* cultures were mixed together into a separate portion of finished compost. After overnight acclimation in the finished compost at room temperature, 100 g of each inoculated compost were combined with separate 10 kg portions of the fresh compost mixture, and thoroughly mixed for 10 min by hand wearing sterile gloves. The inoculated composts were inserted into Tyvek® self-seal pouches (8.89 by 13.33 cm, DuPont, Wilmington, DE) using sterile spoons, and the pouches were placed inside the heaps. Surface compost samples were prepared by placing the composting mixtures in polystyrene trays with vented bottoms, to prevent the collection of water from precipitation events.

2.4. Compost sample placement and temperature and oxygen content measurements

Compost sample bags were placed inside of the compost heaps at the bottom location, measured 30 cm in height from the surface of the concrete slab, at the center of the heap in a circular fashion. The bottom location was chosen for this study because Shepherd et al. (2007) revealed it to be the coldest spot within the temperature stratification inside the compost heaps. Surface compost samples were placed on top of the formed compost heaps and anchored to the compost surface through the use of small bamboo stalks and thin strings.

An OT-21 temperature and oxygen sensor (Demista Instruments, Arlington Heights, IL) was used to measure the ambient oxygen and temperature levels along with those inside of the heaps. In each trial, temperatures and oxygen levels were recorded daily within the first two weeks of composting. Thereafter, temperatures and oxygen contents were recorded at days 21 and 30 in the summer trial and days 21, 30 and 60 in the winter trial.

2.5. Compost sampling and maintenance

Samples were collected on days 0, 1, 3, 5, 7, 14, 21 and 30 in both Trials. An extra sampling at day 60 was included in the winter trial. At each sampling day measurements of temperature and oxygen were recorded before samples were removed from the compost heaps. The samples were transported to the lab and analyzed within 1 h. On days 3, 7, 14 and 21 in the summer trial and days 3, 7, 14, 21, and 30 in the winter trial, the compost heaps were turned mechanically with the use of a front-end loader. After turning, the heaps were manually reconstructed and the sample bags were properly repositioned.

2.6. Microbiological analysis of compost samples

The methods used for the microbial analyses in this study were previously described by Shepherd et al. (2007). Briefly, 25 g of each sample was added to 225 ml of universal pre-enrichment broth (UPB; Acumedia manufacturers Inc., Lansing, MI) in a stomacher bag and homogenized. Serial dilutions of sample homogenates were plated in duplicate on TSA and incubated at 30 and 55 °C