Growth of Seeded *Escherichia coli* in Rewetted Cattle Waste Compost of Different Stages

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ABSTRACT : Compost is used mainly as an organic fertilizer, but it is also used as bedding material for cattle. Dairy cattle have been identified as a main reservoir of pathogenic *Escherichia coli* O157:H7. Further, *E. coli* is regarded as an environmental pathogen that causes bovine clinical mastitis. Hence, its growth in compost spread or compost bedding should be avoided. Physical and chemical conditions, available nutrients and microflora in compost change greatly during the composting process. Since pathogen growth in compost seems to be related to these changes, we assessed the possibility of *E. coli* growth in compost samples collected at 0, 7, 13, 22, 41, 190 and 360 d. Cattle waste composts with and without added tofu residue were collected from static piles and immediately air-dried. Compost samples were inoculated with a pure culture of *E. coli*, the moisture content was adjusted to 50%, and the samples were incubated for 5 d at 30°C. The numbers of *E. coli* in compost before and after incubation were determined by direct plating on Chromocult coliform agar. Almost all compost samples supported *E. coli* growth. Samples collected during or immediately after the thermophilic phase (day 7) showed the highest growth. Growth in samples more than 13 d old were not significantly different from those of aged compost samples. The addition of tofu residue gave a higher growth than its absence in younger samples collected prior to 13 d. To minimize the risk of environmental mastitis, the use of compost in the initial stage of the process is better avoided. ***(Asian-Aust. J. Anim. Sci. 2004. Vol 17, No. 2 : 278-282)***

Key Words : Dairy Cattle, Compost, *Escherichia coli*, Coliforms, Tofu Residue, Thermophilic Phase

INTRODUCTION

Reclamation of organic wastes has been promoted from the viewpoint of environmentally sustainable agriculture. Composting, which can provide marketable products, is a common way to recycle animal wastes. Compost is normally used as an organic fertilizer, but it is also used as a bedding material for cattle.

Dairy cattle have been identified as a principal carrier of pathogenic *Escherichia coli* O157:H7 (Hancock et al., 1994). Wang et al. (1996) demonstrated that O157:H7 could survive for long periods of time in bovine feces. Cattle feces have a relatively high moisture content, making it difficult to reach the thermal death point for pathogens during the composting process without the addition of a bulking agent such as straw or sawdust. Hanajima et al. (2001) demonstrated that the addition of tofu (soybean curd) residue to cattle waste solids raised the temperature to the thermal death point in large parts of the pile at a relatively high moisture content. Co-composting of livestock feces with other waste streams is likely in future owing to its improvement of the process and the trend in the recycling of organic resources.

Bedding material is soiled with urine and feces, wetting it and contaminating it with microorganisms. *E. coli* is a frequently isolated pathogen in cases of clinical mastitis in dairy herds (Hogan et al., 1989; Schukken et al., 1990). Therefore, growth of both mastitis-causing *E. coli* and O157:H7 in bedding material should be avoided. Millner et al. (1987) reported that the types and amounts of microorganisms affect the growth of salmonellae in the compost. Although it is unclear whether these factors could affect *E. coli* or other coliforms, their investigation implies the possibility of biological suppression of the compost.

Where compost is used as bedding material, dryness is important, but the age of the compost has been ignored so far. Attention was also focused the extent of regrowth of *E. coli* in compost used as organic fertilizer. The objective of this study was to assess the possibility of *E. coli* growth in compost samples of different stages. We also compared the growth of *E. coli* in cattle waste compost made with and without tofu residue.

MATERIALS AND METHODS

Compost and sampling

Two static compost piles, one of cattle waste (control) and the other of cattle waste blended with tofu residue (treated), were used for this experiment. The former consisted of a mixture of the solid fraction of separated cow slurry, fresh cow feces, and shredded rice straw (2.2:0.6:1.0 on a dry-matter basis). The latter substituted tofu residue for 15% of the total dry matter. The moisture content of the mixes was adjusted to 78% with tap water, and 220 kg of each material was composted in parallel. During the

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Received June 11, 2003; Accepted October 11, 2003

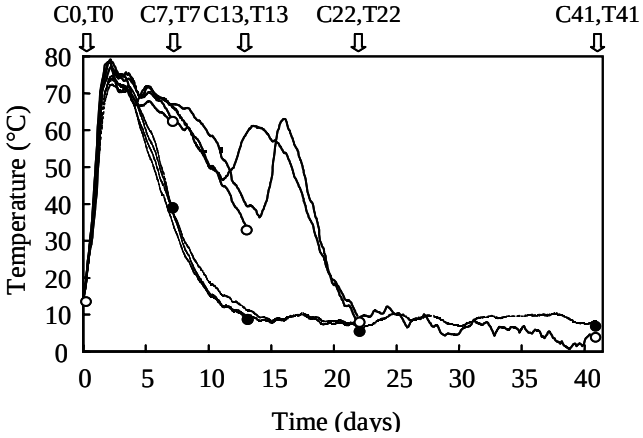

Figure 1. Temperature records at nearly mid-depth of the center in static piles and the time schedule for compost sample collection.
* Thin line: temperature in control pile; thick line: temperature in pile containing tofu residue. Closed and open cirles show the temperatures at sample collection for control and tofu-treated pile, respectively".

composting process, the piles were not turned. The details are described in our previous report (Hanajima et al., 2001). Eight plastic-mesh bags attached with a thermocouple contained about 400 g of mixture were prepared. A total of four plastic-mesh bags were set at nearly mid-depth of the center in each pile, and the compost temperature was measured every hour with a recorder (Thermodac EF Model 5020A, Eto Electrics, Mitaka, Japan). Compost samples were collected by pulling a plastic-mesh bag from the pile, immediately spread on a tray for air-drying until the moisture content was below 20% and thereafter stored in a plastic bag at room temperature until required. Two aged compost samples derived from another composting run (made from a mixture of cow feces and straw), which had been composted and kept in an open plastic bag for 190 or 360 d (referred to as A190 or A360, respectively), were used as well.

Bacterial inoculate

E. coli O157:H37, strain CE273 (calf fecal isolate; nonpathogenic), was obtained from Dr. M. Nakazawa of the National Institute of Animal Health, Japan. The strain was grown in 100 mL of nutrient broth (Difco Laboratories, Detroit, Michigan, USA) for 18 h at 37°C with agitation (150 rpm). The bacteria were sedimented by centrifugation (7000×g, 10 min), washed twice in 0.1 M phosphate-buffered saline (PBS) (pH 7.2), and resuspended in PBS. The concentration was adjusted with PBS to give an approximate inoculation of 2.0×10^8 CFU/mL.

Enumeration

The presence of *E. coli* and other coliforms was tested by plating serial dilutions (1:9) of homogenized composts in triplicate on Chromocult coliform agar (Merck, Darmstadt, Germany). After incubation at 37°C for 24 h, pink colonies resulting from salmon-galactoside cleavage by β-D-galactosidase were classified as coliforms, and dark blue colonies resulting from salmon-galactoside by β-D-galactosidase and X-glucuronide cleavage by β-D-glucuronidase were classified as *E. coli*. The test's high discrimination ability for *E. coli* has been reported in investigations of water and wastewater samples (Frampton et al., 1988; Manafi et al., 1989; Alonso et al., 1998; Byamukama et al., 2000). As a preliminary check, the presumptive *E. coli* colonies from cattle feces and the compost were characterized with an API 20 E test (BioMérieux, Marcy-l'Etoile, France). The test verified all of 50 randomly selected *E. coli* colonies from the plates. Therefore, we considered the Chromocult coliform agar test to be efficient for *E. coli* detection in our experiments.

Incubation

Compost samples used in the incubation test were sieved through a 0.5 cm mesh. Before inoculation, air-dried samples were tested for the presence of indigenous *E. coli* and coliforms. Inoculum of the *E. coli* CE273 was added to 15 g compost samples (dry matter basis; DM) to give a population of 10^7 CFU/g DM, approximately the same level of counts initially present in raw cattle feces, and moisture content was adjusted to 50% with sterilized distilled water. Inoculated samples were thoroughly mixed in a sterilized plastic bag and placed in a 100 mL flask with a sponge plug. The flasks were incubated in a box containing a water beaker at 30°C for 5 d. Simultaneous determination of the number of *E. coli* and coliforms was carried out before and after incubation. All incubation tests were performed in triplicate.

Statistical analysis

The effect of composting stage on the *E. coli* growth calculated as % of initial *E. coli* population was analyzed by one-way analysis of variance. Differences between means were tested by Tukey's multiple range test. All analyses were conducted with the GLM procedure of SAS (SAS Institute, 1988).

RESULTS

Composting process

The average ambient temperature during the composting trial was 4.1°C. The temperature histories of the compost samples are shown in Figure 1. Raw manure (day 0) and compost samples collected on days 7, 13, 22 and 41 in the control and the tofu-treated piles are referred to as C0, C7, C13, C22, and C41 and T0, T7, T13, T22, and T41, respectively. The maximum temperature in each pile

Table 1. Numbers of *E. coli* and coliforms in the air-dried compost and their populations before and after incubation

Compost samples[1]	After air-drying		Inoculation of *E. coli*[2]	After incubation	
	E. coli (CFU/g DM)	Coliforms (CFU/g DM)	*E. coli* (CFU/g DM)	*E. coli* (CFU/g DM)	Coliforms (CFU/g DM)
C0	7.8×10^5	$<10^4$	Average: 2.3×10^7	7.8×10^7	1.2×10^7
C7	$<10^4$	1.7×10^5	Range: 1.8 to 2.8×10^7	3.7×10^8	2.9×10^8
C13	$<10^4$	5.6×10^7	"	3.1×10^8	7.4×10^8
C22	$<10^4$	2.3×10^8	"	8.3×10^6	1.1×10^9
C41	$<10^4$	1.0×10^7	"	2.9×10^7	7.2×10^7
A190	$<10^2$ *	$<10^2$	"	5.7×10^7	$<10^5$
A360	$<10^2$	$<10^2$	"	6.0×10^7	$<10^5$
T0	7.6×10^5	1.3×10^4	"	4.1×10^8	1.2×10^8
T7	5.5×10^4	3.2×10^5	"	5.6×10^8	1.4×10^8
T13	$<10^4$	1.3×10^7	"	2.5×10^8	9.3×10^7
T22	2.2×10^4	5.2×10^6	"	1.5×10^8	9.6×10^7
T41	$<10^4$	2.9×10^6	"	3.7×10^7	4.0×10^7

[1] C=control; T=tofu residue; numbers=age (d) of sample collected; A190 and A360: compost samples derived from another composting run, which had been composted and kept in plastic bags for 190 or 360 d, respectively.
[2] Quantity of *E. coli* inoculated into each air-dried compost sample. * Below limit of detection.

(control, 73.7°C; treated, 78.2°C) was recorded on day 2. The temperature in the control pile gradually decreased to below 10°C after 13 d, but the tofu-treated pile showed a longer thermogenic phase than the control, and its temperature decreased below 10°C on day 22. As we did not expect higher temperature development later, sample collection was terminated on day 41.

Indigenous *E. coli* and coliform populations in compost samples

The populations of *E. coli* and coliforms in the raw material before composting were around 10^7 CFU/g DM. As the compost process proceeded, the numbers in both piles decreased below the detection limit by plate count ($<10^2$ CFU/g DM) through thermophilic composting. During the air-drying treatment, however, a slight regrowth was observed in both treatments. Indigenous fecal bacterial counts in air-dried compost samples used for the incubation test are listed in Table 1. The numbers of *E. coli* in composted samples were below 10^5 CFU/g DM, whereas the number of coliforms tended to be higher. No *E. coli* or coliforms were detected in compost samples that were allowed to stand for 190 or 360 days.

Growth of *E. coli* in compost of different stages

The average initial *E. coli* population in the compost samples after inoculation was 2.3×10^7 CFU/g DM (range, 1.8 to 2.8×10^7 CFU/g DM). This is approximately the same level of *E. coli* counts initially present in raw cattle feces. The growth of inoculated *E. coli* was evaluated by the percentage of *E. coli* counts after incubation to those before incubation. A comparison of *E. coli* growth between different stages of compost samples is shown in Figure 2. High growth more than 1,000% was observed in samples C7 and C13 (control) and T0, T7 and T13 (tofu-treated). Tofu-treated compost tended to have a higher growth in the initial composting stages (days 0 to 13) than the control. The highest growth in each treatment occurred in C7 and T7. The two aged compost samples had slight growth of 275% (A360) and 265% (A190), which were not significantly different. Growth in samples more than 13 d old was not significantly different from those of aged compost samples. C22 was the only sample that showed a decrease in *E. coli* population.

DISCUSSION

For the purpose of investigating the survival of *E. coli* in compost, we inoculated *E. coli* derived from calf feces into compost samples of different stages at an initial population density representative of that in raw cattle waste. Our results show that *E. coli* was not significantly suppressed in most compost samples. Even the aged compost (A190 and A360) showed slight growth. A high growth (>1,000%) was observed mainly in samples in the initial stages of composting. Samples more than 13 d old showed lower growth than younger samples. Russ and Yanko (1981) observed a similar result in their study of salmonellae regrowth in well-composted sludge. They found that the carbon/nitrogen ratio of the compost sample affected the salmonellae growth potential. It is well known that the carbon/nitrogen ratio decreases as the composting process proceeds (Epstein, 1997), so the age of the compost could be a critical factor in pathogen growth.

Compost samples that supported high *E. coli* growth (>1,000%) were collected mainly during or just after the thermophilic phase. In particular, the day 7 samples (C7 and T7) showed the highest growth. Mathur et al. (1993) determined the biochemical oxygen demand (BOD) and the

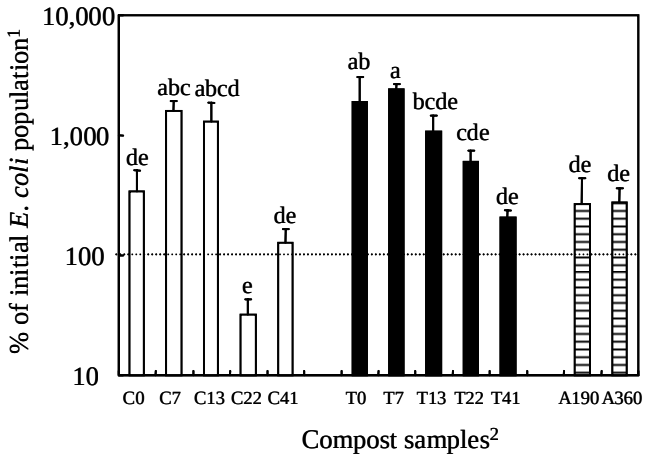

Figure 2. Comparison of *E. coli* growth in compost samples of different stages with and without the addition of tofu residue, and aged compost samples.

[1] % of initial *E. coli* population calculated as [*E. coli* counts after 5 days incubation]/[*E. coli* counts before incubation]×100.

[2] C=control; T=tofu residue; numbers=age (d) of sample collected; A190 and A360: compost samples derived from another composting run, which had been composted and kept in plastic bags for 190 or 360 d, respectively.

* abcde: Means with different letters are significantly different ($p<0.05$). Bars show standard deviation.

dissolved organic carbon (DOC) content of hot-water extracts of compost samples of several ages. They found that the DOC increased as decomposition began, and then declined as the thermophilic phase ended. The results of their BOD determination indicated that the organic matter that was extracted initially and during or immediately after the thermophilic phase was more biodegradable than that extracted in the latter phase of composting. Soares et al. (1995) evaluated *E. coli* regrowth potential in composting facility samples and observed substantial regrowth in two very dry samples. They proposed that low-moisture operation results in the incomplete degradation of the materials and in the availability of nutrients in the final compost, allowing *E. coli* regrowth to occur. Therefore, we assume the growth observed in our compost samples is due to available nutrients and is assumed to be the result of breakdown of compost material structure into easily available fragments, and lysis of thermophiles and other microbes by the cooling and desiccation of the material.

Nutrients in tofu residue accelerated thermogenic composting (Hanajima et al., 2001, Figure 1) and contributed to *E. coli* elimination through heat exposure, while the residue supported *E. coli* growth in rewetted compost. The growth in T0 was significantly higher than that in C0. An enzymatic digestion of soybean meal is often used as a constituent of enrichment medium (e.g., Tryptic soy broth) for *E. coli*. We consider that the *E. coli* used some assimilable nutrients left in the residue for its growth. As the composting process proceeded, however, the difference in growth between treatments became smaller, until finally the growth were the same as in the aged compost samples. Hanajima et al. (2001) showed that the addition of tofu residue increased the BOD, but the value decreased to nearly the same level as without tofu residue during 12 d of composting. Therefore, assimilable nutrients could be consumed in a couple of weeks of thermogenic composting.

The only reduced population was observed in the C22 sample. Although the reason is unclear, it is likely that biological suppression affected growth. Millner et al. (1987) reported that the presence of coliforms only or metabolically active bacteria and actinomycetes resulted in the death of salmonellae in compost. Golueke (1983) noted that indigenous organisms are in a better position to compete for nutrients than pathogenic microorganisms. Indeed, the numbers of coliforms before (2.3×10^8 CFU/g DM) and after (1.1×10^9 CFU/g DM) incubation in the C22 sample were the highest recorded throughout the experiment. The coexistence of a number of coliforms presumably affected the growth of *E. coli*.

Our results reveal that compost collected during or immediately after the thermophilic phase can support *E. coli* growth in the presence of *E. coli* contamination and adequate moisture. Smith et al. (1985) reported that the rate of mastitis infection by coliforms was greatest during summer and coincided with maximum exposure to coliforms in bedding. To minimize the risk of environmental mastitis, the use of compost collected at this stage as bedding is better avoided. In contrast, *E. coli* or coliform populations did not flourish in manure that was composted for 190 or 360 d (Table 1), stable and dry compost is not a favorable habitat for them. Although the inoculated aged compost showed slight growth, the population density after incubation was nearly the same as was inoculated. Stable and dry compost is preferable to younger compost as bedding material for cattle. Furthermore, to minimize contamination by urine and feces, which promote buildup of *E. coli* populations in the bedding material, frequent changes of compost are also important.

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