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Project Title

Environmental effects on the growth or survival of stress-adapted *Escherichia coli* O157:H7 and *Salmonella spp*. In compost

Project Period

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Objectives

Objective 1: Determine the thermal resistance of stress-adapted *E. coli* O157:H7 and *Salmonella* spp. in various types of compost at elevated composting temperatures in a humidity chamber by simulating early stages of on-farm composting.

Objective 2: Apply competitive exclusion microorganisms as secondary treatment to eliminate the regrowth of stress-adapted pathogens in cured compost.

Objective 3: Improve the sensitivity of pathogen detection from compost by combing phage enrichment and Pathatrix® detection system.

Objective 1: Thermal inactivation of *Escherichia coli* O157:H7 and *Salmonella* spp. by simulating early phase of composting:

To simulate what could happen during early phase of on-farm composting, in this study, we used fresh compost mix with two different C:N ratios, i.e. 25:1 (optimal) and16:1 (sub-optimal), which were further adjusted to two moisture levels of 50% (optimal) and 40% (sub-optimal). The thermal inactivation trials were conducted at composting temperatures of 50, 55, and 60^{o}C inside a humidity-controlled environmental chamber with come-up time set as either 2 days (optimal) or 5 days (sub-optimal). At selected intervals, the surviving populations of pathogens were enumerated. Due to the nonlinear reduction of bacterial population during composting, the thermal inactivation data were fit into the mixed Weibull model. Furthermore, the established model was used to predict the pathogen inactivation in compost heaps from our previous on-farm composting trials. The followings are detailed outcomes from this research.

Fresh compost sample preparation: Fresh dairy manure and poultry manure were collected from local farms. Compost with C:N ratios of 16:1 and 25:1 was prepared by mixing above animal wastes with saw dust, hay (carbon source) or wasted feed in different ratios. Fresh compost mix was stored in refrigerator until used. The compost with different moisture contents (40 & 50%) was made by spraying with sterile tap water. Dairy compost with desired C:N ratio and moisture content was inoculated with three-strain mixture of rifampin resistant (Rif^{r}) *E. coli* O157:H7 [Strains F06M-0923-21 (spinach outbreak), F07M-020-1 (Taco John outbreak), avirulent B6914] in 1:100 ratio to a final concentration of ca. 10^{7} CFU/g and mixed continuously for 10 min on sterile polypropylene tray by hand wearing a sterile glove. A three-strain mixture of *Salmonella* spp. (*S*. Typhimurium, *S*. Enteritidis, and *S*. Heidelburg) was inoculated into fresh poultry compost mix in the same way as for *E. coli* O157:H7.

Non-isothermal inactivation study*:* The inoculated compost mixture was put immediately into Tyvek® pouches (5.25 x10 inches) and these bags were then kept in a single layer on the shelves of an environmental chamber with 70% relative humidity. The temperatures used for the study were 50, 55 and 60^{o}C, which were monitored constantly using type-T thermocouples connected to a HotMux data logger. The temperature rise during the study was programmed to ramp step-wise from room temperature to target temperature in 2 days (representing normal temperature rise during composting process) or 5 days (slow heat-up). After temperature of the compost inside the bag reached the target temperature, duplicate sample bags were removed at the predetermined time intervals and cooled immediately in ice water bath. The surviving populations of *E. coli* O157 and *Salmonella* spp. in compost were enumerated on Trypticase soy agar supplemented with100 µg rifampin/ml (TSA/R) and XLT-4 agar with rifampin (XLT-4/R), separately. Two or three trials were conducted for each experiment.

Initially, we compared thermal inactivation of Young culture (YC) (grown in regular Tryptose soya broth, TSB) and Low nutrient-adapted culture (LN) (grown in 1:10 strength of TSB) at different composting temperatures as described in the original proposal. Both YC and LN *E. coli* O157:H7 survived for 72, 24 and 24 h at 50, 55 and 60^{o}C respectively, in compost with 40 and 50% moisture with 2 days of come-up time. The decline in LN population was slight quicker than the YC during 2-day come-up time (Table 1.1). After temperature reached the set point, the population decline was gradual for both YC and LN cultures. Since YC culture survived equally or slight better than LN culture, we decided to use YC for the rest of the composting trials.

E. coli O157 populations were reduced gradually during the come-up time (2 and 5 days) before the inactivation temperature ramped to the selected experimental temperature, with *E. coli* O157 reduction ranging from 0.43 to 1.29 logs. There was a rapid decline in the surviving population of *E. coli* O157 when the target composting temperature was reached (0 h) for all trials. However, the population decline was more in compost with 50% moisture, which was ca. 4.5, 5.7 (enrichment positive) and 5.3 logs at 50, 55 and 60^{o}C respectively, in comparison to reduction of ca. 4.1, 4.8 and 3.9 logs when the moisture was 40% at above temperatures, respectively, with 2 days of come-up time. When the come-up time was 5 days, the pathogen declined more, i.e., ca. 5.51, 5.36 and 5.7 (enrichment positive) logs in compost with 50% moisture than in compost with 40% moisture (4.91, 4.97 and 5.38 logs, respectively). **For all trials, the compost with 50%**

moisture had more reduction in the surviving *E. coli* O157 population than when the moisture was sub-optimal (40%).

In compost with optimal C:N ratio, moisture and come-up time (25:1, 50%, 2 days), YC *E. coli* O157 survived for 72, 24 and 24 h at 50, 55 and 60°C respectively. However, when the come-up time for the same compost was extended to 5 days, the pathogen was detectable through 96, 48 and 48 h at above temperatures, respectively, indicating longer survival of the organism (Table 1.1-3). When moisture content of the same compost was reduced to 40% (25:1, 40%, 2 days), *E. coli* O157 was still detectable till 72, 24 and 24 h with 2 days of come-up time at 50, 55 and 60°C, respectively. However, *E. coli* O157 had extended survival and was detectable until 120, 120 and 72 h when the come-up time for the experiment was extended to 5 days at above temperatures, respectively. **These results suggest the heat-adaptation of *E. coli* O157 during long come-up time at the early phase of composting.**

Compost with sub-optimal C:N ratio (16:1), optimal moisture (50%) supported YC *E. coli* O157 survival till 72 h at 55°C and 24 h at 60°C with 2 days of come-up time, and 96 h at 55°C and 24 h at 60°C with 5 days of come-up time (Table 1.2-3). However, when the moisture of the same compost was lowered to 40%, *E. coli* O157 survived 72 and 48 h with 2 days of come-up time, and 120 and 72 h with 5 days of come-up time at 55 and 60°C, respectively. The impact of moisture level in compost with C:N ratio as 16:1 on the reduction of *E. coli* O157 population during come-up time and at target composting temperatures was the same as in compost with optimal C:N ratio (25:1). **Clearly, both low moisture compost and long come-up time extended the survival of pathogens during composting.**

Overall, compost with lower C:N ratio (16:1) allowed longer survival of *E. coli* O157 than the compost with optimal C:N ratio (25:1) (Table 1.4). In our experimental set-up, the inactivation of microbial population was due to the heat that was produced by the environmental chamber rather than the self-heating from microbial metabolism. In real-world setting, compost with sub-optimal C:N ratio is not supposed to attain high inactivation temperature due to lower microbial metabolism. Therefore, in this study, the difference in compost composition and microbial flora may be the reason for longer survival of *E. coli* O157 in compost with 16:1 C:N ratio.

Table 1.4. Summary of *E. coli* O157:H7 surviving the composting process under different conditions

C:N ratio	Come-up Time (day)	Moisture (%)	Hours survived at 50°C	55°C	60°C
25:1	2	40	72	48	24
	2	50	72	24	24
	5	40	144	120	72
	5	50	96	48	48
16:1	2	40	≥288	72	48
	2	50	240	72	24
	5	40	≥288	120	72
	5	50	≥288	96	24

Our study on thermal inactivation of *Salmonella* in fresh poultry compost revealed that *Salmonella* in compost with 25:1 C:N ratio, 50% moisture and 2 days of come-up time survived until 72 and 24 h at 55 and 60°C respectively (Table 1.5-6). However, in same compost with 40% moisture, *Salmonella* survived 144 and 72 h at 55 and 60°C respectively. Reduction was fast during 24 h before the temperature reached the set level. There was reduction of ca. 3.41 and 3.55 log cfu/g at 55 and 60°C respectively, in compost with 40% and ca. 4.31 and 4.61 log cfu/g at 55 and 60°C respectively, in compost with 50% moisture. **These results suggest that *Salmonella* in poultry compost with sub-optimal moisture (40%) survive longer than in the compost with optimal moisture.**

Fitting the Weibull model with thermal inactivation data collected from the environmental chamber study:
The thermal inactivation pattern of bacteria in compost are not only shown as non-log-linear, but also shown changes of shapes with the changes of the physiological state of the cells. In order to describe the complex behavior of bacteria in compost, we applied a model of mixed Weibull distributions (Coroller *et al.*, 2006) to describe our experimental data. We first fit the mixed Weibull distribution model to the thermal inactivation data of *E coli* O157:H7 in fresh compost under different experimental conditions. There are total 20 experimental series including three composting temperatures: 50, 55 and 60°C; two moisture conditions: 40

and 50%; two C:N ratios: 25:1 and 16:1; and two come-up times: fast (2 days) and slow (5 days). We are able to fit the model to all 12 experimental results under C:N of 25:1 with $R^2 \geq 0.98$. However, we can only fit 4 of 8 experimental results under C:N of 16:1 with $R^2 \geq 0.91$ (Fig. 1.1-2).

Mixed Weibull model was used to fit the survival curve of YC *E. coli* O157 at 50, 55 and 60^{o}C. The results showed that survival curve was non-linear ($p > 1$ or $p < 1$) for all the treatments (Fig. 1.1-2 & Table 1.7-9). In compost with different C:N ratio, moisture and come-up time, δ_2 values were more than their respective δ_1 values at all the temperatures indicating more resistant sub-population resulting in tailing of thermal inactivation curve. It is obvious from the Weibull parameters (Table 1.7-9) that 4 *D*- values for the compost inactivated after 5 days of come-up time was more than the compost inactivated after 2 days of come-up time for most of the treatments, **indicating the microbial population was more resistant when the temperature rise was slow.** Also for most of the treatment 4 *D*- value for the compost with 40% (sub-optimal) moisture was greater than the compost with 50% (optimal) moisture within their respective come-up time.

The regression coefficients of four factors: temperature, moisture, come-up time and C:N ratio were examined for each parameter in the mixed Weibull distribution using the fitting results of those 16 experiments. We used the stepwise regression tool in Matlab to get the regression coefficients. For each regression coefficient, a "t" statistic test is performed to get a *p* value, which can be used to determine whether the variable has statistically significant predictive capability in the presence of the other factors. The results revealed that the C:N ratio is a significant factor for α; the moisture and come-up time are significant for δ_1; the temperature, moisture and come-up time are significant for δ_2; and moisture and come-up time are significant for *p* value (Table 1.7-9. or Fig. 1.3).

***Predicting the thermal inactivation of E. coli O157:H7 during field composting*:** In order to examine the effect of different factors such as temperature, moisture, come-up time and C:N, on the parameters of the mixed Weibull model, the interaction response surfaces were calculated using Matlab. Based on these interaction response surfaces, we first predicted the values of parameters of mixed Weibull model. The temperature we used for prediction is the average of the temperatures during the field trials of previous composting experiments. Then, we applied the mixed Weibull model to predict the survival of *E. coli* O157 population during composting. Overall, for all 4 trials, the pathogen inactivation during early phase of composting was predicted well, with RMSE values between the prediction and experimental results ranging from 0.21 to 1.29. However, there was some discrepancy between the predicted values and actual data after day 7 of composting when the bacterial populations were low, i.e. less than 2 log CFU/g, and the enrichment had to be used for pathogen detection. Since the data from all trials showed the extensive tailing in thermal inactivation curve, this mixed Weibull model may be improved by incorporating the factor of persisters in the compost.

Objective 2: Apply competitive exclusion microorganisms as a secondary treatment to eliminate the regrowth of stress-adapted pathogens in cured compost.

For **Objective 2**, we explored the effect of several environmental factors, such as indigenous microbial populations and water content of compost, and incubation temperature, on the growth of a few *E. coli* O157 or *Salmonella* spp. cells in compost. The competitive exclusion (CE) microorganisms were isolated from the compost samples, and then applied back to the compost as a secondary treatment of enteric pathogens. Furthermore, we evaluated a variety of organic fertilizers for the potential to support the growth of enteric pathogens. The followings are detailed outcomes from this research.

Determining the minimal level of indigenous microorganisms inhibitory to the growth of* E. coli *O157:H7 in finished dairy compost: The commercial dairy compost (Black Kow, moisture content of ca. 40%, background microorganisms of ca. 6.5 log CFU/g) was heat-treated at 80^{o}C for 6 and 18 h to yield ca. 6 and 5 log CFU/g of background microorganisms, respectively. After the moisture was adjusted to 40%, the heat-treated compost was inoculated with a 3-strain mixture of rifampin-resistant *E. coli* O157 at initial concentration of

ca. 3 log CFU/g. The inoculated compost was then incubated at room temperature (ca. 22°C) for 7 days. The plate count data revealed that the population of *E. coli* O157:H7 increased by ca. 2.7 and 3.6 log CFU/g in the compost with ca. 6 and 5 log CFU/g of background microorganisms, respectively, on day 3 and maintained thereafter (Table 2.1). However, in the control compost with ca. 6.5 logs CFU/g of background microorganisms, *E. coli* O157:H7 did not grow at all. **These results suggest that the background microbial population of 6.5 log CFU/g or higher is required to suppress the growth of *E. coli* O157:H7 in the tested compost.** In addition, the predominant background microorganisms in each treatment were identified by 16S rRNA sequencing and GC FAME, showing all predominant cultures belonging to *Bacillus* and *Brevibacillus* spp. The populations of *B. firmus*, *B. aquimaris*, *B. horikoshii* were reduced during heat-treatment, suggesting these microorganisms may be responsible for inhibitory activity against pathogens in the control compost, whereas *Brevibacillus parabrevis*, *B. licheniformis*, and *B. niacini* maintained the similar levels before and after heat treatment.

Table 2.1. Impact of indigenous microorganisms on *E. coli* O157:H7 growth in compost with 40% moisture

Background microorganisms (log CFU/g)	Log CFU *E. coli* O157/g on days of incubation at room temperature						
	0	0.5	1	2	3	5	7
6.5	A* 2.87 ± 0.26	A 2.72 ± 0.19	A 2.75 ± 0.26	A 2.52 ± 0.19	A 2.59 ± 0.13	A 2.11 ± 0.21	A 2.13 ± 0.16
6.0	A 3.07 ± 0.27	B 5.18 ± 0.08	B 5.43 ± 0.29	B 5.59 ± 0.25	B 5.65 ± 0.21	B 5.55 ± 0.13	B 5.28 ± 0.30
5.0	A 3.19 ± 0.19	C 5.89 ± 0.10	C 6.01 ± 0.50	C 6.64 ± 0.20	C 6.82 ± 0.23	C 6.83 ± 0.18	C 6.34 ± 0.34

*Means with different upper case letter in a column are significantly different ($P < 0.05$).

Effect of temperatures on growth of* E. coli *O157:H7 in compost with different levels of background microorganisms: To simulate outdoor conditions throughout the year for storage of finished compost, different temperatures (8, 22, and 30°C) were investigated. *E. coli* O157:H7 did not grow in the compost with ca. 40% moisture and 6.5 logs CFU/g background microorganisms regardless of incubation temperatures. There was also no growth of *E. coli* O157 in the compost with all levels (5, 6, and 6.5 log CFU/g) of background microorganisms when compost was stored at 8°C. With ca. 6 log background microorganisms, maximum increase of *E. coli* O157:H7 population was ca. 2.7 and 2.1 log CFU/g at 22 and 30ºC on day 3, respectively. Similar trend was observed in the compost with ca. 5 log CFU/g background microorganisms with more growth of *E. coli* O157:H7 (Table 2.2). These results suggest that available nutrients in compost could allow rapid growth of both *E. coli* O157:H7 and indigenous microorganisms, whereas *E. coli* O157 levels can be reduced when nutrients became limited at 30°C. Lower incubation temperature (22°C) results in slower growth of mesophilic indigenous microorganisms and may balance the growth of pathogenic microorganisms and the background microflora. **Apparently, *E. coli* O157:H7 can only grow in compost when the outdoor condition is warm, such as in late spring, summer or early fall months.**

Effect of moisture level of compost on* E. coli *O157:H7 growth: The finished compost generally contains moisture ranging from 20 to 40%, and our previous studies suggest that the compost may support the growth of enteric pathogens. Therefore, we made compost with different levels of moisture contents and exposed those to 80°C for 6 or 18 h. Moisture was adjusted to 20, 30, and 40%, followed by inoculation of *E. coli* O157 and incubation at 22°C. *E. coli* O157 grew in the compost with 6 or 5 logs of background microorganisms when at least 20% moisture (a_W of 0.988) was maintained. As moisture content of compost was increased, maximum growth of *E. coli* O157:H7 increased as well (Table 2.3). **These results emphasize the importance to keep the compost as dry as possible during storage in order to control the microbial growth.**

To determine the minimal moisture content of compost and initial level of pathogen required for the growth of *E. coli* O157, the moisture levels of 20, 15, 12.5, and 10%, and inoculum levels of 2, 1, 0.1, and 0.01 log CFU/g were evaluated in the compost with ca. 6 log CFU/g of background microorganisms. As shown in Figure 2.1, *E. coli* O157 grew from ca. 2 to 3 logs in compost with 12.5% moisture (a_W of 0.96), whereas it did not grow in compost with 10% moisture. When moisture content in compost was maintained at 20%, *E. coli* O157 grew from ca. 0.1 to ca. 3 logs (Fig. 2.1-left & middle). Similar growth of *Salmonella* spp. was observed

in the compost with ca. 20% moisture and ca. 6 log CFU/g background microorganisms (Fig. 2.1-right). **Our results clearly indicate that *E. coli* O157 could multiply from a few cells (e.g. 1 cell/g) to hazardous levels in the tested compost at the presence of ca. 6 logs of background microorganisms when minimal moisture content (20%) was maintained.**

***Isolation and identification of CE bacteria against* E. coli *O157:H7 and* Salmonella *spp.*:** Potential CE microorganisms were isolated from various samples including dairy manure-based finished compost, chicken litter-based finished compost, and commercial organic fertilizers. The 786 phenotypically different colonies were purified and tested for the inhibition activity against *E. coli* O157:H7 (Fig. 2.2). For *Salmonella*, we have identified 20 out of 200 isolates having inhibitory activity against three-strain mixture of *Salmonella* spp. After testing for growth rates of potential CE isolates in compost extract and solid compost as compared with individual or mixture of *E. coli* O157:H7, three CE isolates were selected for further study. These CE isolates have been identified as *Brevibacillus parabrevis, Bacillus amyloliquefaciens* and *Pseudomonas thermotolerans* by 16S rRNA method and GC-FAME.

Effectiveness of CE treatment on the growth potential of* E. coli *O157:H7, and* Salmonella *spp. in compost: About 4 logs of CE mixtures were inoculated into compost containing ca. 5 logs of indigenous microorganisms. The inoculated compost was adjusted with sterile tap water to different moisture contents (i.e., 20, 30, ad 40%), and then acclimated at room temperature for 24 h. The 3-strain mixture of *E. coli* O157 was inoculated to the compost next day at an initial concentration of ca. 2 log CFU/g, and then stored at 22 or 30^{o}C. *E. coli* O157:H7 grew in the compost with or without CE application under all conditions (Table 2.4). However, as compared with the controls, the CE treatment was effective by reducing the growth of *E. coli* O157 within 3 days of incubation by 1.1~2.1, 2.2 ~2.6, and 2.6~3.4 logs in compost with moisture levels of 20, 30, and 40%, respectively (Table 2.5). As compared with the control, the growth of *Salmonella* was suppressed by ca. 2.0 logs in CE-treated compost with 40% moisture at 22^{o}C (Table 2.6).

Table 2.5. *E. coli* O157:H7growth reduction in compost of different moisture levels at presence of CE cultures

Temperature (^{o}C)	Compost with moisture content (%) of		
	20	30	40
22	1.05*	2.59	3.36
30	2.12	2.25	2.57

*Growth reduction (log CFU/g) of *E. coli* O157:H7 within 3 days in the compost treated with 4 log CFU/g CE cultures as compared with control compost .

Growth potential of* E. coli *O157 and* Salmonella *in organic fertilizers: We have collected different types of organic fertilizers (n=50) such as animal manure-based compost, super-heated chicken litter pellets, spent mushroom compost, fish emulsions, horse manure compost, bone and blood meals etc. from several states. The growth potential of high populations (ca. 5 log CFU/g) of *E. coli* O157:H7 or *Salmonella* spp. was determined in samples of different nitrogen sources with high water activity ($a_w > 0.98$). Among 10 samples tested, the compost containing fish emulsion as an ingredient, plant-based compost, super-heated chicken litter pellet, and cow manure-based compost allowed the growth of either *E. coli* O157:H7 or *Salmonella* spp. with growth potential ranging from 0.4 to ca. 1 log CFU/g within 2 days of room temperature incubation. These organic fertilizers supporting the pathogen growth contained total aerobic bacterial counts in the range of ca. 5.0 ~ 9.1 log CFU/g, water activity of 0.97 ~ 0.99, and pH of 7.9 ~ 9.0. **These results highlighted the fact that certain types of organic fertilizers may have the potential to support the growth of enteric pathogens due to the composition, physical or biochemical characteristics, and types and levels of indigenous microflora.**

Objective 3: Improve the sensitivity of pathogen detection from compost by combing phage enrichment and Pathatrix® detection system.

The research of Objective 3 involved the isolation of bacteriophages that specifically target the indigenous microflora of compost and manures, and the optimization of detection methods to effectively

inhibit the growth of these organisms to allow for potentially pathogenic organisms to grow at a maximum rate during enrichment.

***Bacteriophage isolation and preparation**:* Enrichments for potential bacteriophage host bacteria were performed using 28 samples of compost and manure in universal pre-enrichment broth (UPB). Colonies were picked following spread-plating of the enrichment media onto TSA, XLT-4, Sorbitol MacConkey Agar (Smac) and Smac supplemented with cefixime (0.05 µg/ml) and potassium tellurite (2.5 µg/ml). Isolates that were in the highest abundance on plates were selected for use in phage isolation. A total of 15 bacterial hosts isolated on TSA were used for phage stock preparation as described below, and twenty-seven bacterial hosts isolated from Smac, CT-Smac and XLT-4 were used for phage cocktail preparation.

The enrichment liquid portion was also used as a stock solution for isolating bacteriophages using the isolated background bacteria as the hosts. The phage particles purified from the enrichment broth were spotted onto TSA agar plates seeded with the host bacteria using standard agar overlay method. Phage solutions that showed positive lysis on the host bacteria were also tested against 10 strains of *E. coli* O157:H7 (ATCC 43890, E0143, E0019, E0654, E0122, E0139, K3999, #286, 0923-21, K262) and 10 strains of *Salmonella* (H3353, DT104, 8243, St. Paul, Dublin, Montevideo, H9301, H9116, Kentucky, Tennessee K4720) to verify that no phages were present that would kill these pathogens being detected. **We have isolated 18 phage stocks, among them 3 stocks contain phages for all 27 host background bacteria.**

***Detection method optimization**:* Considerable efforts and time were spent on the method optimization. A series of experiments were conducted to identify the optimal conditions for the growth of target *E. coli* O15:H7 during enrichment and selective plating, and strong lytic activities of bacteriophage cocktails on the background microorganisms. *E. coli* O157:H7 displayed the maximum growth rate in buffered peptone water (BPW) during enrichment (Fig. 3.1), whereas the bacteriophages had the highest activity in both BPW and UPB (Fig. 3.2). To simulate the impact of bacteriophages on the growth of background microorganisms on agar plates, we overlaid bacteriophage T4 on the surface of spiral-plated compost enrichments containing *E. coli*. Our results showed that high concentrations of phages in a soft agar overlay suppress the growth of *E. coli* significantly, **suggesting agar overlaid with phages can be another approach for pathogen enumeration by plate count methods** (Table 3.1).

Initially, experiments were performed to determine the effectiveness of a phage cocktail at reducing background bacteria levels in compost. Black Kow compost was enriched for 4 and 8 h in three different enrichment media with or without the presence of a phage cocktail (~8 log PFU/ml). There was only slight inhibition ($p>0.05$) of the background microflora by phage treatment. Therefore, our next approach was to test using the compost inoculated with the target *E. coli* O157:H7 strain. A phage cocktail was utilized during the enrichment as well as a second phage treatment prior to sampling. For example, 25 g of Black Kow compost was inoculated with *E. coli* O157:H7 strain 020-1 at a concentration of 1 CFU/g and enriched in 225 ml of UPB supplemented with phage or a buffer for 6 h at 37°C. One milliliter samples were centrifuged and the pellet re-suspended in a buffer or phage cocktail and incubated for 1 h at 37°C, followed by spiral-plating onto CT-Smac or TSA supplemented with rifampin. The results show that the use of two-step phage treatment, one during enrichment and the other prior to sampling, effectively result in the increased numbers of *E. coli* O157:H7 and reduction in background bacteria levels on Smac agar (Fig. 3.3). A similar study using a 10 CFU/g inoculum in fresh dairy compost found that detection of *E. coli* O157:H7 on CT-Smac was only possible when a second bacteriophage treatment was utilized in addition to the enrichment (Fig. 3.4). At the presence of high level of background microorganisms in compost, the identification of positive colonies can be extremely difficult on Smac agar. **Therefore, this two-step phage application during enrichment may aid the enumeration of target pathogens**.

To remain more consistent with currently accepted procedures by Pathatrix® and FDA BAM, BPW was adopted as enrichment media for this phage study. It had already proven previously to provide optimal growing conditions and to lack any inhibition of phage activity. Three trials were performed to test the phage treatment effects on the recovery of *E. coli* O157:H7 inoculated into organic steer manure at a final concentration of ~1 CFU/25 g. Twenty-five gram samples (5 per treatment or control) were enriched in 225 ml of pre-warmed to 42°C BPW with the addition of a phage mixture and incubated at 42°C for 4 h.

Pathatrix® IMS was performed and final bead suspension was plated onto Chromagar. Out of 15 samples tested for the control runs, 10 were positive for *E. coli* O157:H7 on Chromagar plates, and the same number was found to be positive for the treatment plates (Table 3.2). However, the average number of colonies detected on plates that were positive for growth among the controls was 6.75 whereas the average for treatment plates was 27.4, a 4-fold increase in *E. coli* O157:H7 colonies. **This suggests that the use of phages seems to have a positive influence on the likelihood of detecting low levels of pathogen in compost.**

Validating the bacteriophage-assisted pathogen detection method: Screening of compost samples for the presence of *E. coli* O157:H7 was performed in duplicate for both control and treatment. The flow chart for compost sample analysis is presented in Fig. 3.5. Thirty samples have been tested with the pooling method, and 4 using the standard Pathatrix single run trials (Table 3.3). All samples tested so far were negative for the presence of *E. coli* O157:H7. The further validation of this bacteriophage-assisted enrichment method will be completed within a couple months by testing a total of ca. 100 organic fertilizer samples.

Overall outcomes and accomplishments of this project:

***Objective 1*:** Our results have consistently demonstrated that fresh compost with 40% moisture supported better survival of enteric pathogens than the compost with 50% moisture during composting. Come-up time was one of the most critical factors during our composting trials with longer survival being observed for the compost which simulated slow heating process (5 days come-up time) than the one with normal temperature rise (2 days of come-up time) regardless of the moisture level and C:N ratio. The thermal inactivation data for 16 out of 20 experimental series were fit well into the mixed Weibull model, which in turn can predict the inactivation of *E. coli* O157:H7 during early stage of on-farm composting of our previous trials. Both plate count and modeling results suggest that microbial populations get adapted to the composting temperatures well when the temperature rise during come-up time is slow or the composting was conducted under suboptimal conditions. Therefore, for the real-world application, composting process needs to be closely monitored to make sure the rapid temperature rise during early phase of composting.

Objective 2: Our results clearly demonstrate that the finished compost contains sufficient nutrients for a few pathogenic cells to grow under certain conditions such as warm temperatures (≥22^{o}C) and the water activity of compost being maintained at least 0.97. Both the levels and types of indigenous microorganisms play an important role for controlling the pathogen growth in the compost. In the dairy compost tested, the minimal level of 6.5 log CFU indigenous microflora/g was required to suppress the growth of *E. coli* O157 and *Salmonella* spp. By applying the competitive exclusion microorganisms into the compost, the growth potential of *E. coli* O157 and *Salmonella* spp. was reduced ranging from 10 to 2,000 folds as compared with the control. In addition, our results also revealed that the types of organic fertilizers may determine if the enteric pathogens can grow during storage. We believe that our results have some real-world implications. It is important for the produce farmers to store the compost in a dry condition, maintain the sufficient levels of natural microbial flora or add CE cultures in the compost to keep the pathogens from growing, esp. during warm seasons.

Objective 3: Bacteriophage cocktails specific for background bacteria in compost were isolated and characterized. Several approaches to increase the detection sensitivity were attempted. The two-step application of bacteriophages to the enrichment culture resulted in the increased detection of *E. coli* O157:H7 and the reduction of interfering background microorganisms on the selective agar. Although the phage cocktail does not greatly reduce background populations during enrichment, there seems to be some inhibitory effects that are allowing *E. coli* O157:H7 to grow better during 4 ~ 6 h enrichment. Considering that the Pathatrix® procedure is designed to be followed by PCR, a four-fold increase in cell numbers during enrichment can enhance the ability to detect pathogenic bacteria. Since the microbial isolates can be obtained easily, those new isolates can be used to propagate better phage cocktails to target those indigenous microorganisms unique to compost and manure. Therefore, the use of bacteriophage in enrichments to inhibit background interference thus allowing the target pathogen to grow is a method with a great potential.

Describe any collaborative efforts involved in planning and/or implementing this project: Ms. Fernandez-Fenaroli helped us to get in contact with Mrs. Mary Zischke at California Leafy Greens Research Program and Mr. Paul Fleming at Martori Farms to get compost samples for our analysis from Salina, CA, and Arizona, respectively.

Funds-related issues: The fund provided by CPS was adequate for us to carry out the project. Due to large number of compost samples we have collected and used for this project, we allocated the money in supplies category to purchase a 49 cu ft refrigerator with prior approval by UC Davis grant office. The breakdown of the grant funds spent by category is:

Graduate Salaries - $43,138.15
Hourly Salaries - $84,612.30
Fringe Benefits - $26,778.88
Equipment - $13,933.29
Supplies - $47,135.36
Travel - $6,500.00.

Suggestions for future funding by CPS: We enjoyed the close contact with CPS, and all those activities such as the produce farm tour and research symposiums, which helps us to refine our research approaches in order to develop the effective solutions for produce industry. Depending on the nature of each project, it would be helpful for some grants running 2 years instead of 1 year.

Publication list:
Miller, C., J. Kim and X. Jiang. 2010. Microbiological Analysis of Organic Fertilizers and Evaluation of Regrowth Potential for Foodborne Pathogens in the Fertilizers, abstract, ASM Meeting, May 23-27, San Diego, CA.
Kim, J., C. Miller, and X. Jiang. 2010. Indigenous Microorganisms Impacts *Escherichia coli* O157:H7 Growth in Cured Compost, abstract, ASM Meeting, May 23-27, San Diego, CA.
Kim, J., C. Miller, and X. Jiang. 2010. The Regrowth Potential of *Salmonella* spp. in Super-Heated Chicken Litter Pellets, abstract, IAFP Meeting, Aug. 1-4, Anaheim, CA.
Singh, R., J. Kim, F. Luo and X. Jiang. 2010. Thermal inactivation of *Escherichia coli* O157 in fresh compost by simulating field composting process, abstract, IAFP Meeting, Aug. 1-4, Anaheim, CA.

Copies of Publication:

Abstract Title: Indigenous Microorganisms Impacts *Escherichia coli* O157:H7 Growth in Cured Compost (ASM-2010)

Jinkyung Kim, Cortney M. Miller, and Xiuping Jiang, Clemson University, SC, 29634

Composting is a natural biological decomposition process under aerobic and thermophilic conditions. Although proper composting can kill human pathogens, studies have revealed that foodborne pathogens can survive the composting process and then grow in compost and stored biosoilds under favorable conditions. Although suppression pathogens by indigenous microorganisms have been observed in biosolids, live animals or food products, there is a lack of study on the effect of indigenous microorganisms on pathogen regrowth in the cured compost. The dairy manure-based compost was autoclaved for 3 times and then incubated up to 2 days at room temperature to have the target populations of indigenous microorganisms. Three strain-mixture of rifampin-adapted *E. coli* O157:H7 cultures previously grown in the reduced strength of tryptic soy broth was inoculated into above compost with different moistures (20, 30, and 40%) at initial level of ca. 2 log CFU/g, and stored at 8, 22, or 30°C. The microbial community in the compost with different treatments was also compared using denaturing gradient gel electrophoresis. With ca. 5 log CFU background

microorganisms, *E. coli* O157:H7 grew by ca. 5.5 logs in compost with 30 or 40% moisture at 22 or 30ºC, whereas with 20% of moisture, it grew by ca. 2.7 and 4.0 log CFU/g at 22 and 30ºC, respectively. About 6.5 log CFU background microorganisms allowed ca. 4.0 log CFU increase of *E. coli* O157:H7 in compost with at least 30% moisture at same incubation temperatures. In the presence of ca. 8 log CFU/g of background microorganisms, there was ca. 3 log CFU/g of *E. coli* O157 regrowth was observed.
Our results revealed that the level of background microorganisms is critical for the suppression of *E. coli* O157:H7 growth in compost.

Abstract Title: Microbiological Analysis of Organic Fertilizers and Evaluation of Regrowth Potential for Foodborne Pathogens in the Fertilizers (ASM-2010)

Cortney M. Miller, Jinkyung Kim, and Xiuping Jiang, Clemson University, SC, 29634

Many consumers are increasingly demanding organic foods which are produced with the input of organic matter-based fertilizers, which can be a source of human pathogen contamination of fresh produce. Studies revealed that pathogen regrowth could occur from a few cells in dairy compost and biosolids, whereas higher levels of background microflora prevented pathogens from regrowth in cured compost. Therefore, the microbial safety of these products needs to be thoroughly evaluated and ensured. We collected different types of organic nitrogen sources such as animal manure-based compost, super-heated chicken litter pellets, expended mushroom mulch, fish emulsions, bone and blood meals etc., from multiple states. Moisture content, water activity (aw), pH, and microbiological analysis were conducted. The isolates presumptive positive for pathogens were confirmed by PCR or serological test. Moisture contents ranged from 0.98% at the driest to completely liquid samples, and the water activity spanned from 0.298 to 0.999. The average pH was approximately 7.8 with the lowest being 3.6 and the highest being 10.3. The population of background microflora ranged from ca. 3 to 8 log CFU/g. The microbial community was compared using denaturing gradient gel electrophoresis. Some of the samples contained ca. 4~5 log CFU populations of *Enterobacteriaceae* and coliforms. A few samples were positive for *Salmonella*. The regrowth of *E. coli* O157:H7 was evaluated in some fertilizers with high water activity (aw > 0.99) from different sources. The cow manure-based compost supported *E. coli* O157 regrowth by ca. 0.4 log CFU/g within a day. This microbiological information of organic fertilizers can be useful to better understand the microbial ecology in pre-harvest environment.

Title: The Regrowth Potential of *Salmonella* spp. in Super-Heated Chicken Litter Pellets (IAFP 2010)

Authors: Jinkyung Kim, Cortney M. Miller, and Xiuping Jiang

Abstract:

***Introduction*:** Poultry production in the U.S. generates enormous amounts of chicken litter. To better recycle the nutrients in poultry wastes the poultry industry has developed a process to dry the feces with heat and press them into pellets which can then be used as organic fertilizers. Although studies revealed that *Salmonella* regrowth could occur in biosolids and finished composts under favorable conditions, there is no study regarding regrowth potential of *Salmonella* in dried chicken litter pellets.

***Purpose*:** The purpose of this study is to evaluate the possibility of *Salmonella* regrowth in super-heated chicken litter pellets under different environmental conditions.

***Methods*:** Commercially available super-heated chicken litter pellets was acquired and ground into powder. A three-strain mixture of rifampin-adapted *Salmonella* spp. was inoculated into the chicken litter powder (ca. 2 log CFU/g). Moisture was adjusted by fine-misting sterile tap water to desired levels (17-original, 30, 40, and 50%). Samples were stored at 7, 20, and 30°C, and analyzed microbiologically on days 1, 3, 5, and 7.

***Results*:** Original super-heated chicken litter pellets were absent of *Salmonella* spp., *Escherichia coli* O157:H7 and *Listeria monocytogenes* by enrichment. The populations of background microorganisms were ca. 5.4 log CFU/g. At 7°C, *Salmonella* survived through day 7 at all moisture levels, whereas it was detectable up to day 7 at 20°C with only 50% moisture. When the samples were held at 30°C, *Salmonella* died off before day 7. Overall, *Salmonella* did not regrow in chicken litter at any of the moisture levels or storage temperatures. However, *Salmonella* persisted longer in heat-treated chicken litter with high moisture contents at lower temperatures.

Significance: These data suggest that the commercial super-heated chicken litter pellets as organic fertilizers don't support the growth of *Salmonella* spp. However, certain combinations of temperature and moisture level may aid in survival/persistence of the pathogen in chicken litter pellets.

Title: Thermal inactivation of *Escherichia coli* O157 in fresh compost by simulating field composting process

Authors: Randhir Singh, Jinkyung Kim and Xiuping Jiang

Abstract:

Introduction: Thermophilic phase during composting is critical for inactivation/killing of pathogens in animal wastes. However, the persistence and survival of pathogens during composting has been reported.

Purpose: To study thermal inactivation of low nutrient-adapted (LN) *Escherichia coli* O157:H7 in fresh dairy compost by simulating field composting process.

Methods: Three-strain mixture of *E. coli* O157:H7 grown in 1:10 Tryptose soya broth (TSB) for low nutrient adaptation was inoculated into the fresh dairy compost (ca. 10^7 CFU/g) with 40 and 50 % moisture. The culture grown in full strength of TSB served as control. Compost packed in pouches was placed in an environmental chamber (ca. 70% humidity) programmed to ramp from room temperature to selected composting temperatures (50, 55 and 60^{o}C) in 2 days to simulate the early composting phase. The surviving population was analyzed by direct plating or enrichment at predetermined time.

Results: During 2 days of come-up time at early stage of composting, the population of *E. coli* O157 declined gradually. Afterwards, *E. coli* O157:H7 survived for 72, 48 and 24 h in compost with 40% moisture, and 72, 24 and 24 h with 50% moisture at 50, 55 and 60^{o}C, respectively, for both control and LN-adapted cultures. Overall, *E. coli* O157:H7 was inactivated faster in the compost with 50% moisture than the compost with 40% at 55 and 60^{o}C. One day before the end of 2-day come-up period, control *E. coli* O157 survived better (P<0.05) in compost with 40% and 50 % moisture than the LN adapted culture at 50 and 60^{o}C. There was difference (P<0.05) in thermal resistance at 60^{o}C between control and LN-adapted cultures for compost with 40 and 50 % moisture but not at 50 or 55^{o}C.

Significance: Our results suggest that slow come-up time can extend the survival of pathogen during composting. Additionally, both the physiological stage of cultures and the compost moisture level affect the rate of pathogen inactivation as well.

Appendix A
(Objective 1)

Table 1.1. Thermal inactivation of *Escherichia coli* O157 in fresh compost at 50°C under different conditions

Compost C:N ratio	Moisture (%)	Come-up time (days)	Treatment	log CFU/g at 50°C with heating time (h)												
				(-)72	(-) 24	0	2	4	6	8	24	72	96	120	144	168
(25:1)	40	2	YC	NA	6.09	2.9	2.09	2.09	2.07	1.56	(+)	(+)	ND	ND	-	-
			LN	NA	5.5	2.09	1.95	1.98	1.99	1.59	(+)	(+)	ND	ND	-	-
		5	YC	6.92	NS	2.24	2.07	1.96	1.96	1.64	(+)	(+)	(+)	(+)	(+)	ND
	50	2	YC	NA	5.94	2.52	1.98	1.98	1.8	1.56	(+)	(+)	ND	ND	-	-
			LN	NA	5.18	2.17	1.94	1.97	1.7	1.3	(+)	(+)	ND	ND	-	-
		5	YC	6.57	NS	1.49	1.38	(+)	(+)	(+)	(+)	(+)	(+)	ND	ND	-

YC; Young culture, LN; Low-nutrient adapted culture.

NA; Sample not available, NS; Not Sampled, (+); Positive by enrichment, ND; Negative by enrichment

ca. 7 log cfu/g of *E. coli* O157 was inoculated initially in dairy compost

Table1.2. Thermal inactivation of *Escherichia coli* O157 in fresh compost at 55°C under different conditions

Compost C:N ratio	Moisture (%)	Come-up time (days)	Treatment	log CFU/g at 55°C with heating time (h)													
				(-)72	(-) 24	0	1	2	4	8	12	24	48	72	96	120	144
(25:1)	40	2	YC	NA	6.00	2.23	1.93	1.81	1.61	(+)	(+)	(+)	(+)	ND	-	-	-
			LN	NA	5.92	2.03	1.79	1.61	1.59	(+)	(+)	(+)	ND	ND	-	-	-
		5	YC	6.32	NS	2.29	2.03	1.70	1.72	(+)	(+)	(+)	(+)	(+)	(+)	(+)	ND
	50	2	YC	NA	6.02	(+)	(+)	(+)	(+)	(+)	(+)	(+)	ND	ND	-	-	-
			LN	NA	5.95	(+)	(+)	(+)	(+)	(+)	(+)	(+)	ND	ND	-	-	-
		5	YC	5.87	NS	1.64	1.38	1.38	1.41	(+)	(+)	(+)	(+)	ND	ND	-	-
(16:1)	40	2	YC	NA	6.14	4.23	4.08	3.97	3.78	3.64	3.43	(+)	(+)	(+)	ND	ND	-
		5	YC	4.57	NS	3.69	3.52	3.29	3.04	2.36	1.64	(+)	(+)	(+)	(+)	(+)	ND
	50	2	YC	NA	5.94	3.52	3.18	3.02	2.81	2.57	1.77	(+)	(+)	(+)	ND	ND	ND
		5	YC	4.69	NS	2.80	2.37	2.04	1.94	1.80	1.49	(+)	(+)	(+)	(+)	ND	ND

YC; Young culture, LN; Low-nutrient adapted culture.

NA; Sample not available, NS; Not Sampled, (+); Positive by enrichment, ND; Negative by enrichment

ca. 7 log cfu/g of *E. coli* O157 was inoculated initially in dairy compost

Table 1.3. Thermal inactivation of *Escherichia coli* O157 in fresh compost at 60°C under different conditions

Compost C:N ratio	Moisture (%)	Come-up time (days)	Treatment	log CFU/g at 60°C with heating time (h)												
				(-)72	(-) 24	0	0.5	1	1.5	3	4	8	24	48	72	96
(25:1)	40	2	YC	NA	6.40	3.14	3.02	2.86	2.79	2.60	2.21	(+)	(+)	ND	ND	-
			LN	NA	5.88	2.30	2.14	2.03	1.98	1.90	1.52	(+)	(+)	ND	ND	-
		5	YC	5.74	NS	1.62	1.53	1.49	(+)	(+)	(+)	(+)	(+)	(+)	(+)	ND
	50	2	YC	NA	6.40	1.72	1.58	1.65	1.59	1.56	1.60	(+)	(+)	ND	ND	-
			LN	NA	5.53	1.30	1.30	1.44	1.30	1.30	1.30	(+)	(+)	ND	ND	-
		5	YC	5.71	NS	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	ND	ND
(16:1)	40	2	YC	NA	6.25	3.65	3.54	3.32	3.09	2.96	2.78	2.44	(+)	(+)	ND	ND
		5	YC	4.18	NS	2.25	2.10	1.59	(+)	(+)	(+)	(+)	(+)	(+)	(+)	ND
	50	2	YC	NA	5.42	2.61	2.52	2.34	1.97	1.70	1.49	(+)	(+)	ND	ND	-
		5	YC	4.28	NS	1.71	(+)	(+)	(+)	(+)	(+)	(+)	(+)	ND	ND	-

YC; Young culture, LN; Low-nutrient adapted culture.

NA; Sample not available, NS; Not Sampled, (+); Positive by enrichment, ND; Negative by enrichment

ca. 7 log cfu/g of *E. coli* O157 was inoculated initially in dairy compost

Table 1.5. Thermal inactivation of *Salmonella* spp. in fresh compost (25:1, C:N ratio) at 55°C under different conditions.

Moisture (%)	Come-up time (days)	Treatment	log CFU/g at 55°C with heating time (h)										
			(-) 24	0	1	2	4	8	12	72	96	144	168
40	2	Control	3.59	2.76	2.49	2.25	1.89	1.68	1.45	(+)	(+)	(+)	ND
50	2	Control	2.69	2.17	1.41	(+)	(+)	(+)	(+)	(+)	ND	-	-

YC; Young culture, (+); Positive by enrichment, ND; Negative by enrichment

ca. 7 log cfu/g of *Salmonella* was inoculated initially in poultry compost

Table 1.6. Thermal inactivation of *Salmonella* spp. in fresh compost (25:1, C:N ratio) at 60°C under different conditions.

Moisture (%)	Come-up time (days)	Treatment	log CFU/g at 60°C with heating time (h)										
			(-) 24	0	0.5	1	3	4	8	24	48	72	96
40	2	Control	3.45	2.60	2.51	2.47	1.87	1.79	1.45	(+)	(+)	(+)	ND
50	2	Control	2.39	1.95	1.68	(+)	(+)	(+)	(+)	(+)	ND	ND	ND

YC; Young culture, (+); Positive by enrichment, ND; Negative by enrichment

ca. 7 log cfu/g of *Salmonella* was inoculated initially in poultry compost

Table 1.7. Parameters of mixed Weibull distribution of *E. coli* O157:H7 inactivation curves at 50°C

compost C:N ratio	Moisture (%)	Come-up time (days)	Treatment	□*	□ₚh	□□ (h)	p	4*D*- (h)	R^2
(25:1)	40	2	YC	5.39	24.92	127.01	2.23	47.52	0.986
			LN	5.13	18.75	112.31	1.70	43.20	0.984
		5	YC	5.68	78.61	278.06	3.75	115.20	0.990
	50	2	YC	5.40	22.67	124.50	2.06	44.64	0.989
			LN	5.24	15.95	116.12	1.46	41.76	0.984
		5	YC	5.87	51.80	246.41	3.42	77.76	0.989

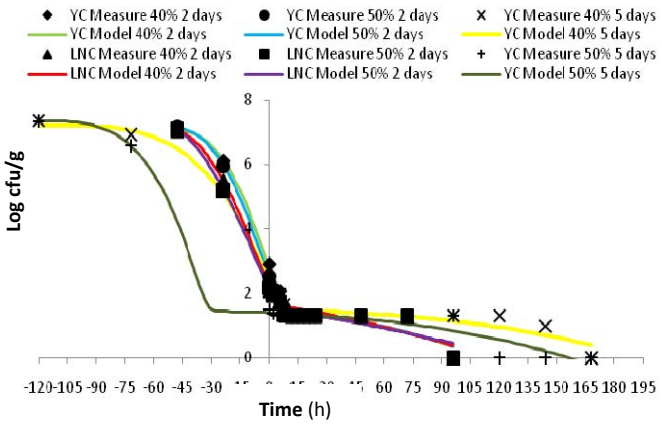
*For mixed Weibull model, α is model parameter, p is the shape parameter and δ_1 and δ_2 are the decimal reduction in subpopulation 1 (sensitive subpopulation) and subpopulation 2 (resistant subpopulation), respectively.

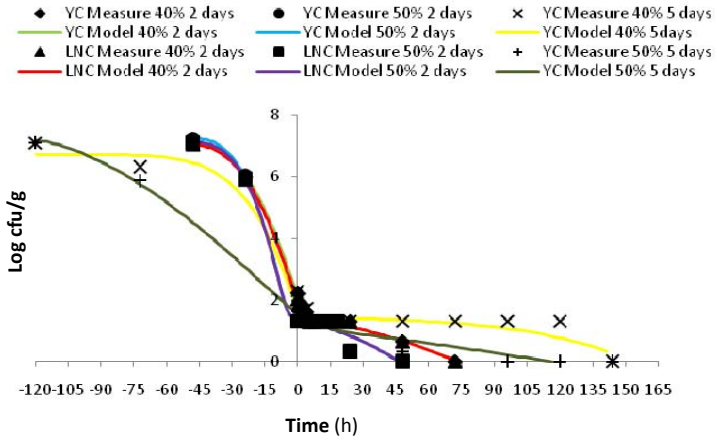
Table 1.8. Parameters of mixed Weibull distribution of *E. coli* O157:H7 inactivation curves at 55°C

Compost C:N ratio	Moisture (%)	Come-up time (days)	Treatment	α	δ_1(h)	δ_2 (h)	p	4*D*- (h)	R^2
(25:1)	40	2	YC	5.48	23.55	97.94	2.28	48.96	0.998
			LN	5.40	23.25	97.62	2.28	44.16	0.998
		5	YC	5.30	93.04	1260.60	6.00	62.64	0.985
	50	2	YC	5.61	22.20	78.62	2.65	42.24	0.993
			LN	5.50	22.56	78.90	2.68	38.40	0.993
		5	YC	5.43	37.95	163.30	1.53	79.92	0.983
(16:1)	40	2	YC	5.70	30.01	662.60	2.08	50.40	0.976
		5	YC	4.49	103.80	259.69	6.00	51.84	0.919
	50	2	YC	5.25	21.91	102.87	1.69	43.20	0.995
		5	YC	4.64	3.57	5.18	0.46	51.84	0.963

Table 1.9. Parameters of mixed Weibull distribution of *E. coli* O157:H7 inactivation curves at 60°C

Compost C:N ratio	Moisture (%)	Come-up time (days)	Treatment	α	δ_1(h)	δ_2 (h)	p	4*D*-(h)	R^2
(25:1)	40	2	YC	5.19	30.65	80.59	3.03	44.40	0.996
			LN	4.95	23.65	71.67	2.24	43.20	0.996
		5	YC	5.58	44.03	193.50	3.98	118.80	0.998
	50	2	YC	5.54	24.26	74.51	2.54	38.40	0.991
			LN	5.28	19.32	69.64	2.06	38.40	0.993
		5	YC	5.15	34.02	123.08	1.66	95.04	0.994
(16:1)	40	2	YC	5.11	24.41	84.24	1.93	59.04	0.996
		5	YC	2.43	1.04	1.09	0.36	132.00	0.964
	50	2	YC	5.20	18.48	77.06	1.65	50.40	0.985
		5	YC	7.11	1.73	3.50	0.41	76.56	0.983





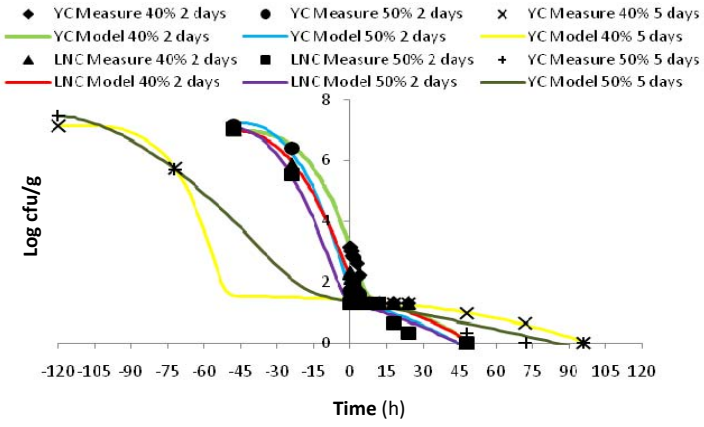


Figure 1.1. Fitted survival curve of mixed Weibull distributions of *E. coli* O157:H7 under different conditions. (top left panel) at 50^{o}C, 25:1 C:N ratio, 2 & 5 days of come-up time; (central panel) 55^{o}C, 25:1 C:N ratio, 2 & 5 days of come-up time; (bottom Panel) 60^{o}C, 25:1 C:N ratio, 2 & 5 days of come-up time; (bottom left) 55^{o}C, 16:1 C:N ratio, 2 & 5 days of come-up time; (bottom right) 60^{o}C, 16:1 C:N ratio, 2 & 5 days of come-up time; Lines are fitted curve of the.

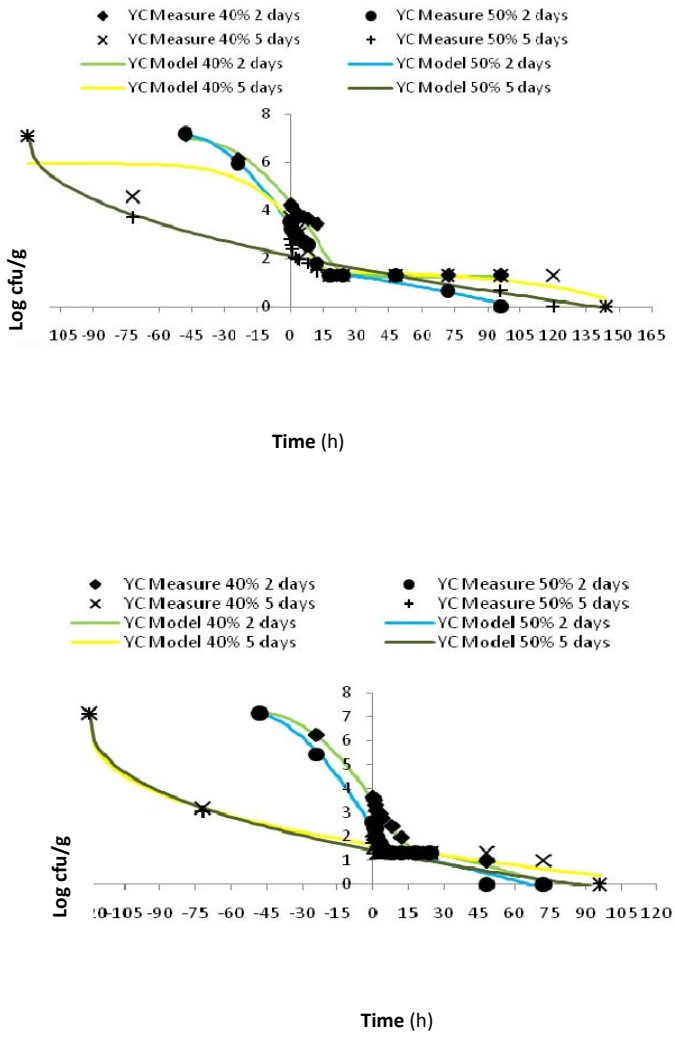


Figure 1.2. Fitted survival curve of mixed Weibull distributions of *E. coli* O157:H7 under different conditions. ((top left panel) 55^{o}C, 16:1 C:N ratio, 2 & 5 days of come-up time; (bottom panel) 60^{o}C, 16:1 C:N ratio, 2 & 5 days of come-up time; Lines are fitted curve of the.

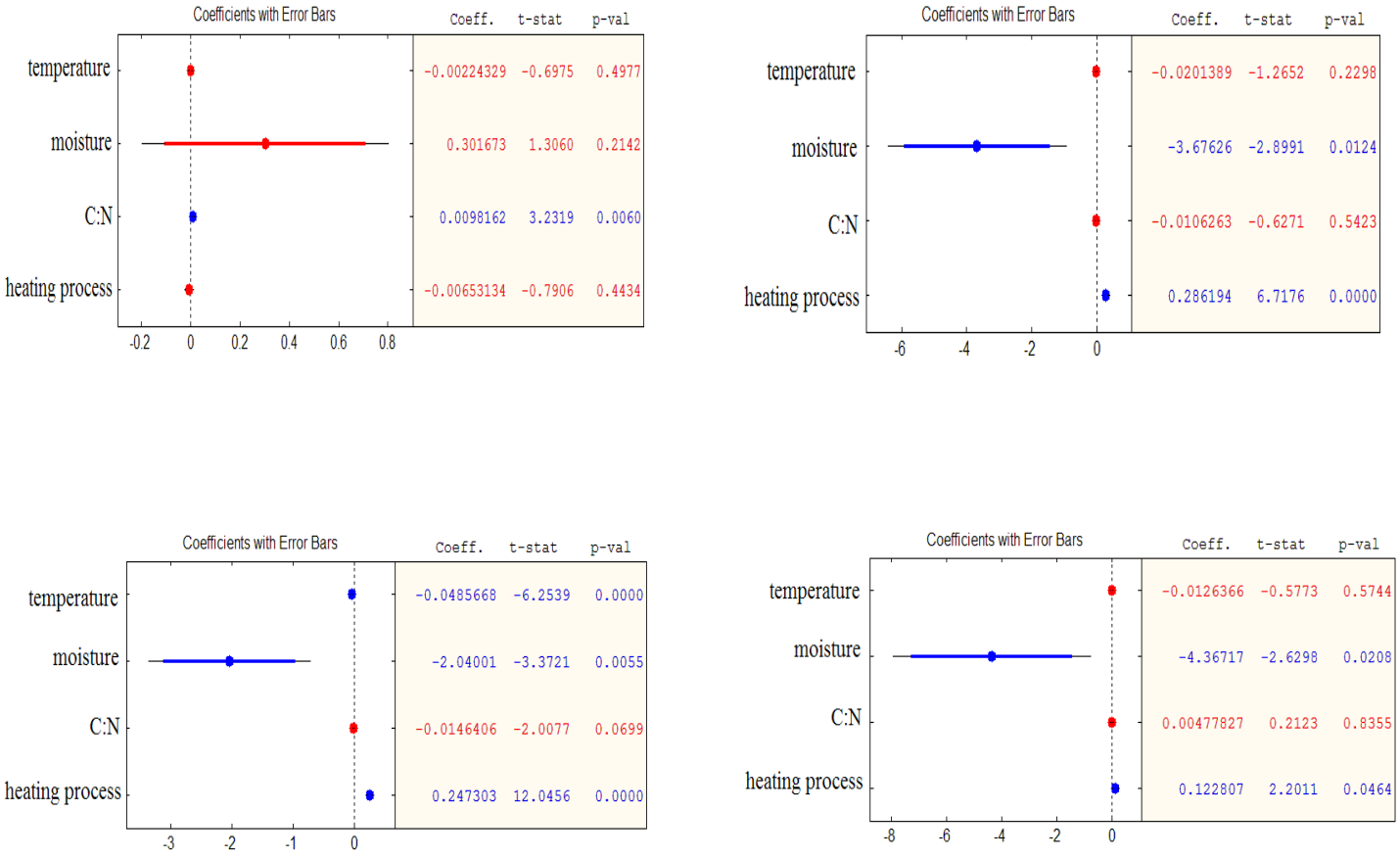


Figure 1.3. Regression Coefficients of four factors; temperature, moisture, come-up time and C:N for different parameters in mixed Weibull model; (top left panel) for parameter α; (top right panel) for parameter δ1; (bottom left) for parameter δ2; (bottom right) for parameter *p*.

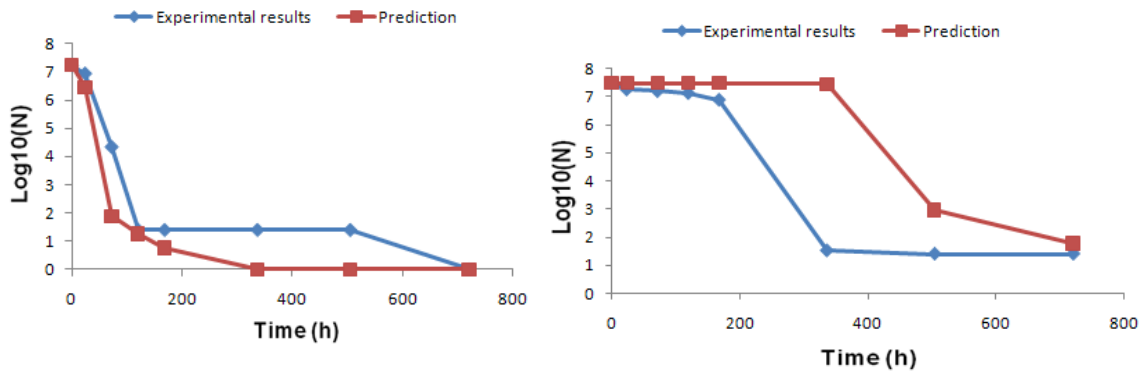


Figure 1.4. Comparison of prediction and experimental results of inactivation of E coli under (left panel). C:N=17:1, moisture 50%, average temperature 47.22 and fast come-up. The RMSE between the prediction and experimental results is 1.23; (right panel) C:N=17:1, moisture 50%, average temperature 31.67 and slow come-up. The RMSE between the prediction and experimental results is 2.34.

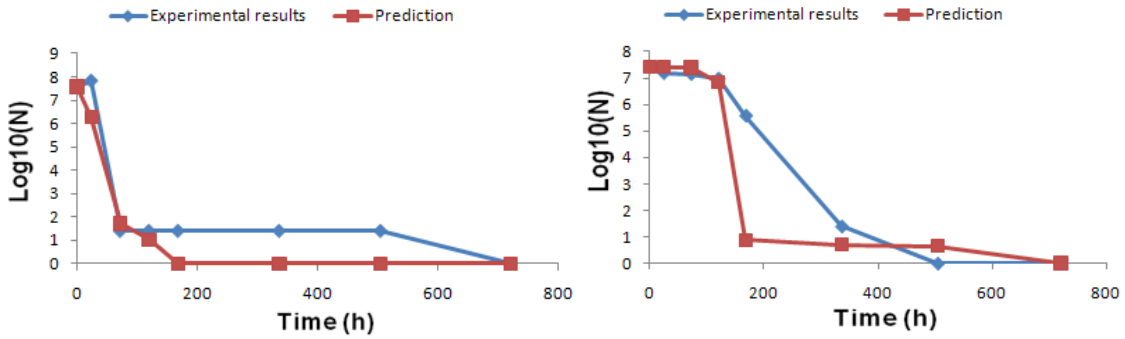


Figure 1.5. Comparison of prediction and experimental results of inactivation of E coli under (left panel) C:N=25:1, moisture 50%, average temperature 49.86 and fast come-up. The RMSE between the prediction and experimental results is 1.10; (right panel) C:N=25:1, moisture 50%, average temperature 31.67 and slow come-up. The RMSE between the prediction and experimental results is 1.82.

Appendix B
(Objective 2)

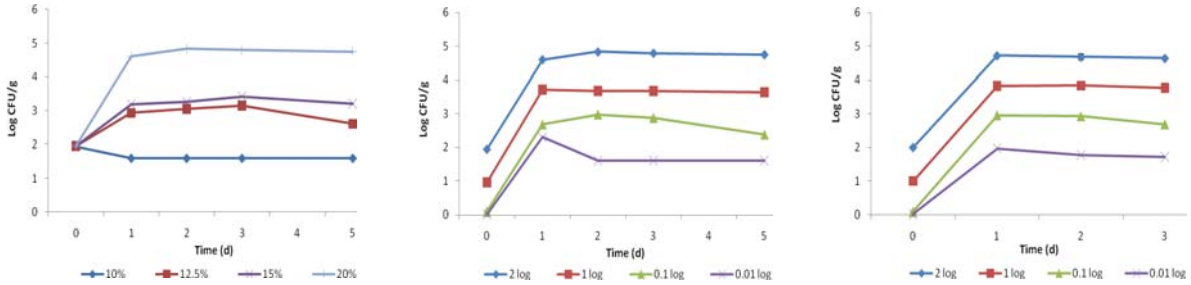


Fig. 2.1. *E. coli* O157:H7 growth in compost with different moistures (left) and inoculum levels (middle) and *Salmonella* growth in compost with 20% moisture at different inoculum levels (right). Detection limit was 1.6 log CFU/g.

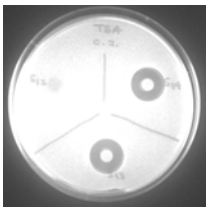


Fig. 2.2. Inhibition zone of competitive exclusion microorganisms against *E. colli* O157:H7 in agar spot assay.

Table 2.2. *E. coli* O157:H7 growth in compost with 40% moisture at different levels of background microorganisms as affected by different incubation temperatures.

Temp (ºC)	Background microorganisms (log CFU/g)	log CFU *E. coli* O157/g on days of incubation						
		0	0.5	1	2	3	5	7
8	6.5	A*2.85 ± 0.17a**	A2.88 ± 0.23ab	A2.98 ± 0.11ab	A2.71 ± 0.13b	A2.86 ± 0.13b	A2.65 ± 0.12c	A2.66 ± 0.20b
	6.0	B3.25 ± 0.04a	AB3.10± 0.07a	A3.08 ± 0.05a	B3.01 ± 0.17a	AB2.91 ± 0.08a	B2.92 ± 0.08a	A2.71 ± 0.2 a
	5.0	B3.22 ± 0.10a	B3.18 ± 0.19a	B3.20 ± 0.09a	B3.06 ± 0.09a	B3.03 ± 0.06a	B2.84 ± 0.16a	A2.94 ± 0.21a
22	6.5	A2.87 ± 0.26a	A2.72 ± 0.19a	A2.75 ± 0.26a	A2.52 ± 0.19a	A2.59 ± 0.13a	A2.11 ± 0.21a	A2.13 ± 0.16a
	6.0	A3.07 ± 0.27a	B5.18 ± 0.08b	B5.43 ± 0.29b	B5.59 ± 0.25c	B5.65 ± 0.21c	B5.55 ± 0.13c	B5.28 ± 0.30c
	5.0	A3.19 ± 0.19a	C5.89 ± 0.10b	C6.01 ± 0.50b	C6.64 ± 0.20c	C6.82 ± 0.23c	C6.83 ± 0.18c	C6.34 ± 0.34c
30	6.5	A2.85 ± 0.17a	A3.10 ± 0.18b	A3.08 ± 0.30b	A2.88 ± 0.11c	A2.85 ± 0.30b	A2.38 ± 0.27b	A2.20 ± 0.37a
	6.0	B3.25 ± 0.04a	B5.36 ± 0.03c	B5.33 ± 0.02b	B5.24 ± 0.06b	B4.70 ± 0.15b	B3.99 ± 0.52b	B3.51 ± 0.11b
	5.0	B3.22 ± 0.10a	C6.10 ± 0.06c	C6.34 ± 0.11c	C6.41 ± 0.19b	C6.03 ± 0.13b	C5.12 ± 0.56b	C4.45 ± 0.15b

*Means with different upper case letter in a column at the same temperature are significantly different ($P < 0.05$).
**Means with different lower case letter in a column with the same level of background microorganisms at different temperature are significantly different ($P < 0.05$).

Table 2.3. *E. coli* O157:H7 growth in compost with different moisture levels.

Moisture content (a_W)	Background microorganisms (log CFU/g)	log CFU *E. coli* O157/g on days of incubation at room temperature						
		0	0.5	1	2	3	5	7
20%	6.0	A*2.77 ± 0.17a**	A4.28 ± 0.00a	A5.49 ± 0.02a	A5.34 ± 0.04a	A5.03 ± 0.48a	A5.20 ± 0.12a	A5.35 ± 0.23a
(0.988)	5.0	A2.56 ± 0.11a	B4.89 ± 0.00a	A5.58 ± 0.18a	A5.47 ± 0.18a	A5.39 ± 0.06a	B5.35 ± 0.01a	A5.32 ± 0.06a
30%	6.0	A3.04 ± 0.10a	A5.68 ± 0.08c	A5.72 ± 0.03a	A5.76 ± 0.04b	A5.77 ± 0.02b	A5.73 ± 0.03c	A5.14 ± 0.17a
(0.995)	5.0	A2.97 ± 0.17b	B5.93 ± 0.02b	B6.05 ± 0.05a	B6.22 ± 0.09b	B6.26 ± 0.06b	B6.24 ± 0.06b	B5.87 ± 0.07b
40%	6.0	A3.07 ± 0.27a	A5.18 ± 0.08b	A5.43 ± 0.29a	A5.59 ± 0.25ab	A5.65 ± 0.21b	A5.55 ± 0.13b	A5.28 ± 0.30a
(0.998)	5.0	A3.19 ± 0.19b	B5.89 ± 0.10b	B6.01 ± 0.50a	B6.64 ± 0.20c	B6.82 ± 0.23c	B6.83 ± 0.18c	B6.34 ± 0.34c

*Means with different upper case letter in a column at the same moisture content are significantly different ($P < 0.05$).
**Means with different lower case letter in a column with the same level of background micoorganisms at different moistureare significantly different ($P < 0.05$).

Table 2.4. *E. coli* O157:H7growth in compost with different moisture levels in the presence of CE cultures at 22 and 30°C

Temperature (°C)	Moisture (%)	CE Treatment	log CFU *E. coli* O157/g on days of incubation at room temperature			
			0	1	2	3
22	20	Control	A*2.30 ± 0.00a**	B4.69 ± 0.01a	B4.52 ± 0.00a	B5.00 ± 0.10a
		Treatment	A2.30 ± 0.00a	A3.64 ± 0.09a	A3.55 ± 0.17a	A3.95 ± 0.19a
	30	Control	A2.30 ± 0.00a	B7.13 ± 0.17c	B8.43± 0.04b	B8.46 ± 0.03b
		Treatment	A2.30 ± 0.00a	A5.40 ± 0.04b	A5.73± 0.11c	A5.87 ± 0.09c
	40	Control	A2.30 ± 0.00a	B6.67 ± 0.17b	B8.52 ± 0.00b	B8.50 ± 0.07b
		Treatment	A2.30 ± 0.00a	A5.46 ± 0.07b	A5.15 ± 0.11b	A5.14 ± 0.14b
30	20	Control	A2.30 ± 0.00a	B5.98 ± 0.09a	B6.84 ± 0.02a	B6.54 ± 0.10a
		Treatment	A2.30 ± 0.00a	A4.42 ± 0.19a	A4.50 ± 0.02a	A4.42 ± 0.06a
	30	Control	A2.30 ± 0.00a	B8.28 ± 0.09b	B8.43 ± 0.03b	B8.50 ± 0.09b
		Treatment	A2.30 ± 0.00a	A5.90 ± 0.07b	A5.90 ± 0.12b	A6.25 ± 0.11c
	40	Control	A2.30 ± 0.00a	B8.72 ± 0.24c	B8.52 ± 0.00b	B8.48 ± 0.05b
		Treatment	A2.30 ± 0.00a	A6.29 ± 0.18c	A6.17 ± 0.03b	A5.91 ± 0.04b

*Means with different upper case letter in a column at the same temperature and moisture content are significantly different ($P < 0.05$).
**Means with different lower case letter in a column at the same temperature and same treatment are significantly different ($P < 0.05$).

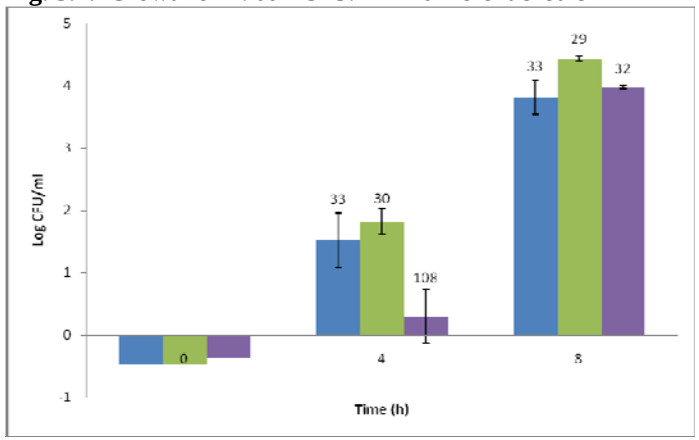
Table 2.6. *Salmonella* growth in compost with 20% moisture content in the presence of CE cultures at 22°C

CE Treatment	log CFU *Salmonella*/g on days of incubation at room temperature			
	0	1	2	3
Control	A*2.24 ± 0.09a**	B5.72 ± 0.05b	B6.81 ± 0.04c	B6.90 ± 0.34c
Treatment	A2.42 ± 0.17a	A5.05 ± 0.11b	A5.07 ± 0.06b	A5.15 ± 0.13b

*Means with different upper case letter in a column are significantly different ($P < 0.05$).
**Means with different lower case letter in a row are significantly different ($P < 0.05$).

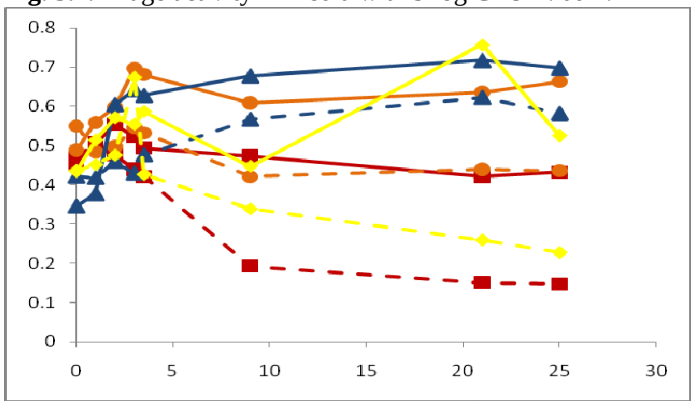
Appendix C
(Objective 3)

Fig. 3.1. Growth of *E. coli* O15:H7 in different broths



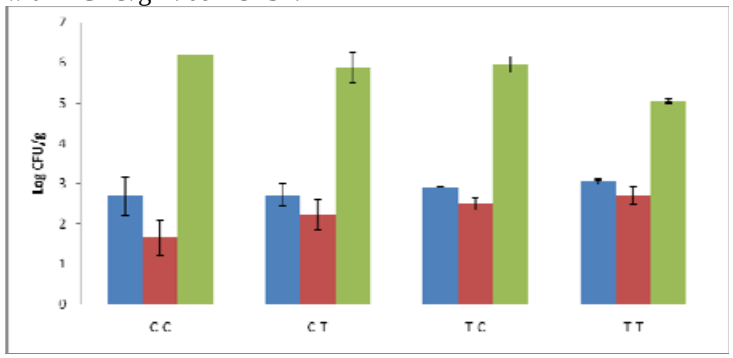
Blue bar, brilliant green bile (BGB); green bar, buffered peptone water (BPW); purple bar, universal pre-enrichment broth (UPB). *Doubling time.

Fig. 3.2. Phage activity in media with 9 log CFU *E. coli* /ml



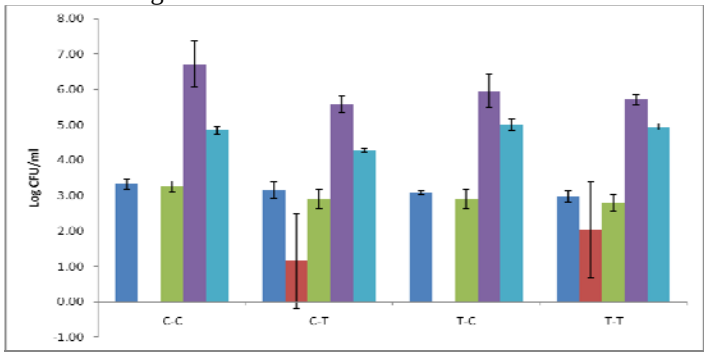
Symbols: ▲, Brilliant Green Bile; ●, modified TSB; ◆, Universal Pre-enrichment broth; ■, Buffered Peptone Water. Control (—) and phage treatment (- - -).

Fig. 3.3. Two-step phage enrichment using Black Kow compost inoculated with 1 CFU/g *E. coli* O157:H7



Blue bar, TSA supplemented with rifampin (100 µg/ml); Red bar, Sorbitol MacConkey agar; Green bar, Background bacterial numbers.
C-C: enrichment with buffer followed by normal sampling
C-T: enrichment in buffer followed by phage addition
T-C: enrichment in phage followed by normal sampling
T-T: enrichment in phage followed by phage addition

Fig. 3.4. Two-step phage enrichment using fresh dairy compost inoculated with 10 CFU/g *E. coli* O157:H7



Blue bar, TSA supplemented with rifampin (100 µg/ml);
Red bar, Sorbitol MacConkey supplemented with cefixime (0.05 µg/ml) and potassium tellurite (2.5 µg/ml) (CTSMAC);
Green bar, Chromagar O157 supplemented with cefixime and potassium tellurite (CTChro);
Purple bar, background bacteria on CTSMAC;
Turquoise bar, background bacteria on CTChro.

Table 3.1. Average number of *E. coli* B colonies on TSA following phage T4 overlay

CFU per plate	Control	Phage 10^6/ml	Phage 10^7/ml	Phage 10^8/ml
120000	TNTC	TNTC	1±4.8	1±1
12000	TNTC	TNTC	3	1±1.4
1200	TNTC	TNTC	519	10±7.6
120	116±10.8	83±40	25±28	0±0.6
12	11±3	7±4.9	4±2	2±1.3

Table 3.2. Plate counts of *E. coli* O157 on Chromagar O157from bacteriophage-assisted Pathatrix IMS inoculation trials

Trial 1		Trial 2		Trial 3	
Cont.	Treat.	Cont.	Treat.	Cont.	Treat.
0, 1	0, 0	0, 0	2, 2	5, 5	47, 47
0, 1	0, 0	1, 4	13, 11	3, 0	0, 0
0, 0	0, 0	0, 0	4, 6	0, 1	139, 108
0, 2	60, 45	63, 45	20, 38	0, 1	0, 1
0, 0	0, 0	0, 0	2, 1	2, 1	0, 2
(3/5)*	(1/5)	(2/5)	(5/5)	(5/5)	(4/5)

*Number of positive samples

Fig. 3.5. Flow chart of compost sample analysis using bacteriophage-assisted enrichment followed by Pathatrix IMS and Chromagar methods

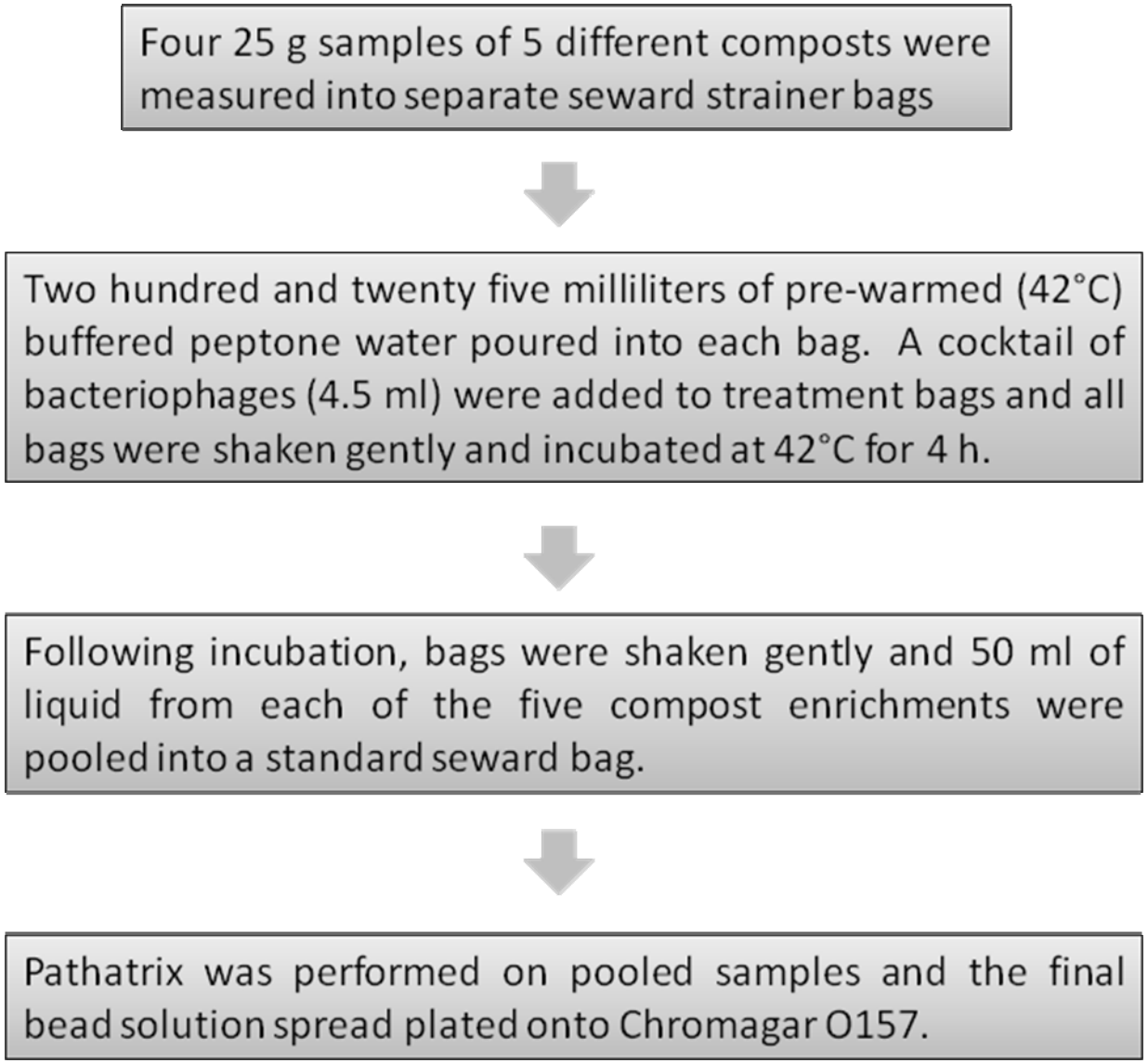


Table 3.3. Summary of compost sample analysis by bacteriophage-assisted enrichment and Pathatrix IMS detection

Sample	Moisture Content (%)	Water Activity (a_w)	pH	Total Bacterial Count (log CFU)	Enterobacteriacae (log CFU)	Coliforms (log CFU)	E. coli (log CFU)	*E. coli* O157	*Salmonella* spp.	Method
3 -Pepper Field Fertilizer (under plastic)	12.16 ± 0.43	1.002 ± 0.00	6.77 ± 0.21	5.79 ± 0.16	1.30 ± 0.00	<1	<1	–	–	P
4 - Pepper Field Fertilizer (uncovered)	8.72 ± 0.84	0.998 ± 0.00	6.65 ± 0.06	6.01 ± 0.13	2.43 ±0.98	2.04 ± 0.06	<1	–	–	P
5 - Eggplant Roots	2.82 ± 0.11	0.986 ± 0.00	6.81 ± 0.21	6.29 ± 0.16	3.66 ± 0.21	3.58 ± 0.16	<1	–	–	P
6 - Eggplant Side Field	0.98 ± 0.01	0.869 ± 0.03	6.77 ± 0.19	6.11 ± 0.16	2.35 ± 1.01	1.00 ± 0.00	<1	–	–	P
10 - True 1200 (feather meal)	8.92 ± 0.17	0.583 ± 0.01	6.22 ± 0.02	3.38 ± 1.02	<1	<1	<1	–	–	P
11 - Greenwaste and horse manure (5 months)	18.68 ± 0.01	0.941 ± 0.00	9.22 ± 0.13	7.02 ± 0.15	<1	<1	<1	–	–	P
12 - Greenwaste and dairy manure	28.04 ± 0.61	0.962 ± 0.01	9.49 ± 0.09	6.23 ± 0.23	<1	<1	<1	–	–	P
14 - Greenwaste and fish emulsion	19.32 ± 0.09	0.942 ± 0.00	9.35 ± 0.04	5.39 ± 0.18	<1	<1	<1	–	–	P
15 - Az, 98% dairy cow manure, 2% sorghum silage, aged 6 months	24 ± 0.85	0.796 ± 0.02	10.15 ± 0.06	4.53 ± 0.29	<1	<1	<1	–	–	P
16 - Az, 98% dairy cow manure, 2% sorghum silage, combined pile ready	5.86 ± 0.23	0.298 ± 0.01	10.29 ± 0.06	6.27 ± 0.09	<1	<1	<1	–	–	P

for spreading aged 6-9 months										
17 - USDA/BARC, Food waste compost: leaves, wood chips, biodegradable plate scrapings, food prep and bio based plates, cups, and cutters, very mature, 3 years	63.99 ± 0.89	1.001 ± 0.00	7.92 ± 0.16	7.00 ± 0.05	5.13 ± 0.18	<1	<1	–	–	P
18 - USDA/ BARC, Aphis yard waste compost, leaves, grass, wood chips, 3 years, mature pile	33.43 ± 0.91	0.994 ± 0.00	7.41 ± 0.04	6.91 ± 0.04	2.71 ± 0.34	1.68 ± 0.28	<1	–	–	P
19 - USDA/BARC manure compost, mixed animal, mostly dairy cow solids and straw, mature, 6 months old	66.27 ± 4.20	1.003 ± 0.00	8.62 ± 0.13	7.11 ± 0.08	4.24 ± 1.94	1.35 ± 0.49	<1	–	–	P
20 - USDA/BARC, greenhouse trimmings and potting soil compost, mature, 3 years old	40.22 ± 5.52	0.998 ± 0.00	8.72 ± 0.06	7.06 ± 0.06	2.50 ± 0.43	1.54 ± 0.19	<1	–	–	P
21 - Lobster Compost	54.59 ± 1.90	1.007 ± 0.01	8.05 ± 0.03	5.35 ± 0.50	<1	<1	<1	–	–	P
22 - New York compost	72.65 ± 0.56	1.006 ± 0.00	8.37 ± 0.06	5.64 ± 0.18	1.24 ± 0.34	1.00 ± 0.00	<1	–	–	P

23 - USDA ARS ANRI EMSL	26.13± 0.30	0.825± 0.013	9.67±0. 04	5.62 ± 0.63	<1	<1	<1	–	–	P
26 - Espoma Plant-tone	8.56± 0.12	0.562± 0.018	6.78±0. 05	5.92 ± 0.16	<1	<1	<1	–	–	P
27 - Espoma Bio-tone Starter Plus	12.05± 0.47	0.640± 0.013	6.96±0. 00	6.06 ± 0.14	<1	<1	<1	–	–	P
28 - TN-Horse and deer based manure	63.36± 1.96	0.998± 0.003	7.38±0. 11	7.76 ± 0.07	5.84 ± 0.13	3.10 ± 0.08	<1	–	–	P
29 - TN-Zoo manure + wood + black sand	64.23± 0.10	1.006± 0.002	8.46±0. 06	7.28 ± 0.03	3.54 ± 0.02	1.77 ± 0.10	<1	–	–	P
31 - GA-Sugar hill Composted Cow Manure	36.54± 0.74	0.999± 0.001	8.04±0. 11	7.76 ± 0.03	3.78 ± 0.53	<1	<1	–	–	P
33-Hi-yield Bone Meal	2.18±0.3 0	0.225± 0.02	7.14 ± 0.11	2.36 ± 0.32	<1	<1	<1	–		S
34-Hi-yield Blood Meal	9.33±0.1 5	0.419± 0.04	7.83 ± 0.01	4.50 ± 0.45	<1	<1	<1	–	–	P
37-Earthsafe Organics Herb Food	4.76±0.2 3	0.405± 0.05	5.94 ± 0.08	5.26 ± 0.38	<1	<1	<1	–		S
38-Fafard Composted Cow Manure	41.40± 3.10	1.000± 0.00	8.19 ± 0.18	7.11 ± 0.41	4.41 ± 0.17	3.26±0.05	<1	–		P
39-Going Green Organics Fertilizer	11.65± 0.05	0.640± 0.03	8.04 ± 0.00	5.29 ± 0.21	<1	<1	<1	–	–	P
40-Barky Beaver Mushroom Compost Mix	62.31± 0.54	0.985± 0.00	9.02 ± 0.01	8.38 ± 0.03	1.15 ± 0.21	1.00 ± 0.00	<1	–		S
41-Feather Meal CCCC	12.01± 0.33	0.631± 0.20	5.83 ± 0.02	5.31 ± 0.46	4.43 ± 0.09	3.57±0.53	<1	–		S
42-Mushroom Compost After Steaming	66.25± 2.09	0.983± 0.00	8.42 ± 0.08	8.07 ± 0.15	3.26±0.22	1.60±0.21	<1	–		P

45-Mushroom Compost Prior to pasteurization	59.97± 10.37	0.981± 0.00	8.06 ± 0.08	9.10 ± 0.13	4.98 ± 0.03	4.53 ± 0.47	<1	–		P
48-Horse Compost (3 months)	61.02± 0.16	0.997± 0.00	9.00 ± 0.02	7.18 ± 0.20	4.08 ± 0.11	3.77 ± 0.15	1.00 ± 0.00	–		P
49-Horse Compost #2 (4 months)	50.58± 1.04	0.989± 0.01	8.09 ± 0.26	7.52 ± 0.08	5.49 ± 0.36	5.19 ± 0.21	<1	–		P
51-EB Stone Organics Tomato and Vegetable Food	3.69±0.33	0.311± 0.07	6.43 ± 0.03	4.84 ± 0.20	2.36 ± 0.79	2.29 ± 0.05	<1	–	–	P
52-EB Stone Organics Steer Manure	33.01± 0.21	0.927± 0.01	9.35 ± 0.09	8.00 ± 0.17	<1	<1	<1	–		P
53-EB Stone Organics Flower and Vegetable Planting Mix	46.70± 0.66	0.995± 0.00	8.67 ± 0.30	7.42 ± 0.06	3.66 ± 0.27	3.54 ± 0.20	<1	–		P

P, pooling; S, individual sampling.