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Survival of pathogenic bacteria in compost with special reference to *Escherichia coli*

GONG Chun-ming[1,2], Koichi Inoue[1], Shunji Inanaga[2] and Takashi Someya[1]

(1. Laboratory of Soil Microbiology, Faculty of Agriculture, Saga University, Saga 840-8502, Japan. E-mail: kd23057@edu.cc.saga-u.ac.jp; 2. The United Graduate School of Agricultural Sciences, Kagoshima University, Kagoshima 890-0065, Japan; 3. The School of Health Sciences, University of Occupational and Environmental Health, Kitakyushu 807-8555, Japan)

Abstract: Application of compost in agricultural practice could potentially cause contamination of foodstuffs with pathogenic bacteria such as *Escherichia coli* O157:H7 (*E. Coli* O157). We investigated pathogenic bacteria in compost collected from the compost facilities, and evaluated the survival of *E. coli* K12 and O157 in laboratory experiments. Out of 19 compost product samples, coliform bacteria and salmonella were detected in 7 and 3 samples respectively. The number of coliform bacteria was 1.8×10^2 to 2.5×10^6 CFU/g dw and that of salmonella was 4.2×10^1 to 6.0×10^3 CFU/g dw. Moreover, coliform bacteria, fecal coliform, *E. coli* and salmonella were detected during composting at 54℃ to 67℃. The results indicated that moisture content was a very important factor to the heat sensitivity of pathogenic bacteria in compost, *E. coli* in compost of high moisture content was more sensitive than that in compost of low moisture content, cells harvested in logarithmic phase was more sensitive than these in stationary phase, and *E. coli* K12 was more sensitive than *E. coli* O157. Based on the D values, the lethal time of *E. coli* K12 and O157 from 10^8 to 10^9 CFU/g dw were 16.3 and 28.8 min, respectively, at 60℃ in compost with 40% moisture content. However, some *E. coli* cells survived in composting process at 54℃ to 67℃. Water potential (low moisture content) and physiological aspects of bacteria (stationary phase) could explain only in part of the prolonged survival of *E. coli* in compost, and there should be some other factors that are conducive to bacterial survival in compost.

Keywords: survival; pathogenic bacteria; compost; *Escherichia coli*; moisture

Introduction

Increasing public interests have been concerned on safety foods and the environmental aspects recently, especially on the so-called organic foods, which are made from crops grown with organic fertilizers or compost but not chemical fertilizers. Public concerns on organic products have accelerated the use of compost even to vegetables that are usually consumed without heat processing. Compost made from animal waste and other organic refuse can serve as a valuable nutrient resource to the agricultural fields, and decrease environmental load if they are treated properly.

However, it has been reported that several foodborne outbreaks of bacterial infections were associated with the consumption of raw fruits and vegetables contaminated by manure (Cieslak, 1993; Chapman, 1997; Itoh, 1998; Little, 1999). Among human pathogens associated with compost, *E. coli*, especially that of serogroup O157:H7 (*E. coli* O157), could be the most serious menace, since infection with *E. coli* O157 has become an emerging problem worldwide (Coia, 1998; Konuma, 2000). Although most strains of the bacterium *E. coli* are nonpathogenic and considered to be part of the normal microbial flora of the gastro-intestinal tract of man and other warm blooded animals, some vero cyto toxin-producing strains including *E. coli* O157 can cause diarrhea, haemorrhagic colitis or haemolytic uremia syndrome. It has emerged as a foodborne pathogen of considerable public health importance and has been implicated in over 20000 cases of infection and 250 deaths per year in U. S. (Finelli, 1995; Koutkia, 1997), and 700 cases in UK (Anon, 1995). Outbreaks of food poisoning by *E. coli* O157 in Japan were first recorded in 1996, when several large outbreaks occurred. Among them, the outbreak in Sakai City, was serious and presumably caused by contaminated radish sprouts, affected nearly 8000 people, mostly school children, and resulted in three death (Michino, 1999). The Sakai affair led us to recognize the importance of biosafety control of agricultural products that are usually consumed without heating.

Outbreaks have been traced to ground beef, raw milk, water, unpasteurized apple cider and vegetables that have been contaminated by this pathogen (Pell, 1997). Cattle, sheep and deer are considered the main animal reservoirs of this pathogen (Keene, 1997; Tauxe, 1997). Wang *et al.* (Wang, 1996) found that *E. coli* O157 remained viable in bovine feces for up to 70 d, depending on the concentration level and the temperature. Effective control of this microorganism in bovine feces will minimize its contamination of the environment and human food. Hence, it is important to examine the number of coliform bacteria and the survival characteristics of *E. coli* in compost environment. However, in Japan, there is neither criterion about the health standard of compost, nor the method of inspecting the pathogenic microbes in compost.

Foodborne outbreaks of *E. coli* O157 associated with the consumption of raw fruits, vegetables and their unpasteurized products have been linked to the use of compost or fecal contamination during the growing, harvesting, processing, or distribution of these commodities (CDC, 1997). Until now, there have been many studies on the research of *E. coli* O157, most of them were focused on the study of *E. coli* in soil, aquatic environment and organic waste (Shere, 1998; Kudva, 1998; Wang, 1998; Gafliardi, 2000; Maule, 2000). There are only a few studies on the survival of *E. coli* O157 in compost (Lung, 2001). The objectives of the study were to investigate pathogen bacteria in compost collected from the compost facilities, and to evaluate the survival of *E. coli* K12 and O157 in laboratory experiments.

1 Materials and methods

1.1 Compost sample

The compost samples were collected from the compost facilities of various parts of West Japan during September, 1997 to March, 2004, all together was 25 samples, including 19 compost products, 3 uncompleted compost and 3 raw materials. The raw materials to be composed were cow dung,

chicken droppings, kitchen garbage, sewage sludge and food residues. Wooden chipping were also mixed in the compost, which was used as dressing in a barn. Compost samples(each 500 g) were collected at five different places, at 30 to 50 cm depth from the surface of a compost pile. These samples were well mixed and about 500 g of the mixture was taken and sealed in a plastic bag, which was kept in a cooler and carried to the laboratory. All samples were analyzed in laboratory within three days after collection.

1.2 Stains media and cultivation

Each compost sample(10 g wet weight) was added to sterile saline (0.85% NaCl) solution (95 ml), and homogenized at 15000 r/min for 15 min with a homogenizer (NISSEI AM-3, Japan), then 10-fold serial dilution was made with sterile saline solution to obtain dilutions of 10^{-1} to 10^{-5}. The population density was determined by the culture methods.

Pathogenic bacteria, such as coliform bacteria, fecal coliform, *E. coli* and salmonella were determined by the dilution plate counts (DPC) method or the most probable number(MPN) method. Their media, cultural and conditions are shown in Table 1.

Table 1 Media and cultural conditions of indicator pathogenic organisms

Organism	Medium	Method	Condition	Criteria
Coliform bacteria	DCA	DPC	37℃, 1 d	Red colony
Fecal coliform	EC	MPN	45℃, 1 d	Air bubbles, acidification of medium
E. coli	CCA	DPC	37℃, 1 d	Dark blue colony
Salmonella	MLCB	DPC	37℃, 1 d	Black colony

Notes: DCA. desoxycholate agar(Eiken, Japan); EC. *E. Coli* broth(Eiken, Japan); CCA. chromocult coliform agar(Merck, Germany); MLCB. mannitol L-lysine crystalviolet brilliantgreen agar(Nissui, Japan).

Cell numbers of pathogenic bacteria were determined by DPC or MPN methods. For DPC methods, three aliquots(100 μl) of each dilution(10^{-1}, 10^{-3} and 10^{-5}) were inoculated triplicate plates. Plates containing 25 to 250 colonies were enumerated and recorded as colony forming units (CFU) per gram of the dry weight, after incubation at 37℃ for 24 h. For MPN method, five aliquots(1 ml) of each dilution(10^{-1} to 10^{-5}) were inoculated into the medium (10 ml) in quintuplicate test tubes (15 × 10^5 mm). The growth of bacteria was observed after incubating at 45℃ for 24 h, and the number of bacteria was calculated.

The composition of cultural medium, DCA medium contained 10.0 g peptone, 10.0 g lactose, 1.0 g sodium desoxycholate, 5.0 g sodium chloride, 2.0 g dipotassium hydrogen phosphate, 2.0 g gouyuansuan iron ammonium, 0.033 g neutral red and 15.0 g agar, pH 7.3 in a liter distilled water. EC medium contained 20.0 g peptone, 5.0 g lactose, 1.5 g bile salts, 4.0 g dipotassium hydrogen phosphate, 1.5 g potassium dihydrogen phosphate, and 5.0 g sodium chloride, pH 6.9 in a liter distilled water. CCA medium contained 3.0 g peptone, 5.0 g sodium chloride, 2.2 g sodium dihydrogen phosphate, 2.7 g disodium hydrogen phosphate, 1.0 g pyruvate sodium, 1.0 g tryptophan, 1.0 g sorbitol, 0.4 g coloring matter, and 10.0 g agar, pH 6.8 in a liter distilled water. MLCB medium contained 10.0 g peptone, 5.0 g yeast extract, 2.0 g heart extract, 4.0 g sodium chloride, 3.0 g mannit, 5.0 g L-lysine chloride salt, 4.0 g sodium thiosulfate, 1.0 g gouyuansuan iron ammonium, 0.0125 g brilliant green, 0.01 g crystal violet, and 15.0 g agar, pH 6.8 in a liter distilled water.

1.3 Heat sensitivity of *E. coli* K12 and O157 in saline and compost

1.3.1 Adjustment of compost moisture centent

The collected cow dung compost was dried at 105℃ for 10 h in an oven, passed through 1 mm mesh sieves, put into a reagent bottle, sterilized for 15 min at 121℃ in an autoclave, and finally stored at 4℃ until use. In this experiment, sterile distilled water was added into compost samples before bacterial inoculation, to obtain compost of 70% and 40% moisture content.

1.3.2 Bacterial strains

The strain of *E. coli* K12 was used as a laboratory and a dlinical of *E. coli* O157 strain was isolated at the University of Occupational and Environmental Health (Kitakyushu, Japan). These strains were grown in fresh nutrient broth (Eiken, Japan) for 18 and 24 h at 37℃ to reach logarithmic phase(OD_{660}, 0.35) and stationary phase(OD_{660}, 0.50) cells. Then they were washed with centrifugation(12000 r/min, 10 min) at least twice with sterile saline, and suspended in the same solution. At the same time, the suspension was plated out on nutrient agar(Eiken, Japan) and incubated at 37℃ overnight.

1.3.3 Inoculation and bacterial enumeration

E. coli K12 or O157 suspension (100 μl) at a concentration of 10^9 CFU/ml was inoculated into sterile saline solution(0.9 ml) and compost of 70% and 40% moisture content(0.9 g) in vails, which were immersed in a water bath at 60℃ for 0, 1, 2, 3, 6 and 10 min. Sterile saline(9 ml) was added into each heat treated sample, which was homogenized and diluted by the DPC method. The suitable dilution was inoculatd on nutrient agar and incubated at 37℃ for 18—24 h. The colony number was counted and the number of survival *E. coli* was calculated.

1.3.4 *D* value

Decimal reduction time(DRT), is the necessary time for reducing density to 1/10 of the initial population. It can be calculated by the following equation(Yanagita, 1981):

$$k = \frac{1}{t} \cdot \frac{\log N_0 - \log N}{0.434}. \quad (1)$$

Where, k is a coefficient of reduction speed, N_0 is the initial bacteria density and N is the bacteria density after time t. When t is equal to D, this equation can be changed to Equation (2) (Yanagita, 1981), expressed as:

$$D = 2.0303/k. \quad (2)$$

The necessary time from the initial density to be destructed completely can be calculated by D value.

2 Results and discussion

2.1 Pathogenic bacteria in compost

Out of the 19 compost product samples, coliform bacteria and salmonella were detected from 7 and 3, respectively. The number of coliform bacteria was 1.8×10^2 to 2.5×10^6 CFU/g dw and salmonella was 4.2×10 to 6.0×10^3 CFU/g dw(data not shown). Among the 7 compost samples where coliform bacteria were detected, four were derived from cow dung, two were derived from sewage sludge and food residues, the other one was from kitchen garbage.

As for samples in which salmonella were detected, two were from cow dung, and the other one was from kitchen garbage. The results showed that pathogenic bacteria were destructed in most of compost after composting process, however, some pathogenic bacteria still survived in a part of compost product derived from various organic refuse. Similar results have been obtained by Soares *et al*. (Soares, 1995) and Sciancalepore *et al*. (Sciancalepore, 1996).

It was reported that moisture, carbon availability and microbial diversity were the main parameters affecting pathogen regrowth in compost (Deportes, 1995). Temperature, pH and NaCl were controlling factors to the growth of *Salmonella enteritidis* and *E. coli* O157 (Blackburn, 1997). The survival of coliform bacteria in compost may be ascribed to the heterogeneity of temperature in compost piles, or the inappropriate monitor of other controlling factors.

2.2 Pathogenic bacterial number in raw material, uncompleted compost and compost product

In order to investigate survival of pathogenic bacteria in composting process, the compost samples were collected from two composting facilities(Saga, Japan), where cow dung and garbage were used as raw material. The numbers of pathogenic bacteria in raw material (0 d), uncompleted compost(30 d) and compost product(60 d for cow dung manure and 50 d for garbage compost) are listed in Table 2.

Table 2 Detection of coliform bacteria, fecal coliform, *E. coli* and salmonella in raw material, uncompleted compost and compost product

Raw material	Composting period, d	Temp. ℃	Number of pathogenic bacteria, CFU or MPN/g			
			Coliform bacteria	Fecal coliform	*E. coli*	Salmonella
Cow dung	0	26	8.1×10^{10}	1.8×10^{10}	8.5×10^{7}	ND[b]
	30	67	7.2×10^{8}	7.0×10^{8}	ND[b]	ND[a]
	60	42	7.6×10^{5}	3.1×10^{4}	ND[a]	ND[a]
Garbage	0	48	$>10^{6}$	$>10^{6}$	$>10^{5}$	$>10^{7}$
	30	54	9.2×10^{4}	9.2×10^{4}	ND[b]	3.7×10^{4}
	50	44	2.4×10^{3}	2.4×10^{2}	ND[a]	6.0×10^{3}

Notes: a. Not detected($<10^{1}$ CFU/g dw); b. not detected($<10^{2}$ CFU/g dw)

The number of coliform bacteria was 10^{10} and $>10^{6}$ CFU/g dw in raw material of cow dung and garbage, respectively, and which fell to 10^{5} and 10^{3} CFU/g dw in the product after composting. The number of fecal coliform, *E. coli* and salmonella showed similar trend during composting process. Specially, it should be noted that some pathogenic bacteria survived in uncompleted compost though the temperature of composting process was 54—67℃.

In general, pathogenic bacteria are destructed very rapidly at high temperature. However, the investigation for detecting coliform bacteria, fecal coliform, *E. coli* and salmonella of the samples collected from deeper sites of compost piles revealed the survival of these pathogenic bacteria in sites where temperature was 54℃ or higher(Table 2). This finding is consistent with the results reported by other authors. Droffner *et al*. (Droffner, 1995) reported salmonella and *E. coli* survived for 59 d at about 60℃ in industrial compost, and reported that the *E. coli*, *S. typhimurium* and *P. aeruginosa* mutants were able to grow even at 54℃ (Droffner, 1991a; 1991b). All these studies indicated that the number of pathogenic bacteria survived even under high temperature for a long period. Under current regulations in U. S., in-vessel and aerated static-pile composting should ensure operating conditions of 55℃ or higher for at least 3 d to attain adequate pathogen destruction (CFR, 1998), and for the inactivation of pathogens in composting, composting should ensure operating conditions of 55℃ for 3 d or 50 to 60℃ for at least 7 d for municipal solid waste and manure (EPA, 1980); the finished class A compost should have fecal coliform of $\leqslant1000$ most probable number per g of dried solids or $\leqslant3$ salmonella per 4 g dried solids. The criteria may be insufficient based on the above findings. As for Japan, there is no regulation or standard for compost operation concerning hygiene control.

2.3 Survival of *E. coli* K12 and O157 in different condition

The heat sensitivity of *E. coli* K12 and O157 in sterile saline solution or compost at 60℃ are shown in Fig.1. In the case of *E. coli* K12 in logarithmic phase, the initial number was 10^{8} CFU/g dw that fell to 10^{0} CFU/ml after about 2 min in sterile saline solution and the number reduction was a little slower in compost of 70% moisture content. Whereas, in compost of 40% moisture content, cells of 10^{3} CFU/g dw survived even after heat treating for 10 min(Fig.1a). Similar results were observed on cells in the stationary phase. Whereas, the heat resistance of *E. coli* K12 was stronger than that in logarithmic phase for conditions examined(Fig. 1b). The trend of *E. coli* O157 was similar to *E. coli* K12, except that the destruction was slower than that of *E. coli* K12(Fig.1c, 1d). As a result, *E. coli* in compost of high moisture content is more sensitive to heating than that of lower moisture content, cells in logarithmic phase is more sensitive than that in stationary phase, and *E. coli* K12 is more sensitive than *E. coli* O157. The above results showed that moisture content should be a very important effect factor to the survival of *E. coli* in compost, and implied that compost may serve *E. coli* better environment for survival than aqueous solutions at high temperature.

Based on the death curve shown in Fig.1, *D* values of *E. coli* K12 and O157 are calculated and listed in Table 3.

D value of *E. coli* in compost of 40% moisture content was much higher than that in saline and compost of 70% moisture content, *D* value of cells in stationary phase was higher than that in logarithmic phase, and *D* value of O157 was higher than that of K12. These results showed that *D* value of *E. coli* O157 was about 3 times higher and that of K12 was 3 to 6 times higher in compost of 40% moisture content than in that of 70% moisture content. In brief, this study indicated that lower moisture content and physiological state (stationary phase) could improve the resistance of *E. coli*, suggesting that as well as temperature, it is also important to control moisture content appropriately in composting process.

Table 3 *D* value of *E. coli* K12 and O157 in saline and compost at 60℃

Media	*D* value, s			
	Logarithmic phase		Stationary phase	
	K12	O157	K12	O157
Saline	15.0	17.2	22.1	30.4
Compost(70% moisture content)	15.4	40.0	29.0	61.6
Compost(40% moisture content)	109.9	136.4	122.4	215.9

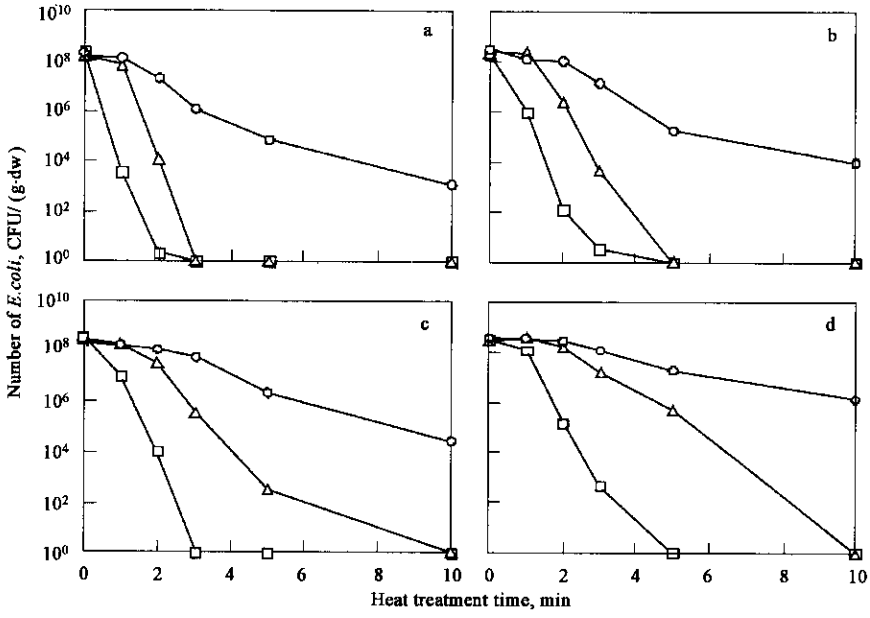

Fig. 1 Death curves of *E. coli* K12 and O157 at 60℃ in saline, compost of 70% and 40% moisture content
a. *E. coli* K12 in logarithmic phase; b. *E. coli* K12 in stationary phase; c. *E. coli* O157 in logarithmic phase; d. *E. coli* O157 in stationary phase; ---□---saline solution; ---△--- compost(70% moisture content); ---○---compost(40% moisture content)

Based on the laboratory experiments, the time decrease from 10^8 to 10^0 CFU/g dw, calculated from the acquired *D* value, would be 16.3 min for *E. coli* K12 and 28.8 min for *E. coli* O157 in stationary phase at 60℃ in compost of 40% moisture content. It was also reported that the lethal temperature and necessary time to destroy *E. coli* ranged from 15 to 20 min at 60℃ and 1 h at 55℃ (Deportes, 1995). Therefore, theoretically, all *E. coli* cells in compost at temperature above 55℃ should be dead after several hours. However, in fact, some *E. coli* cells survived during the composting even under temperature as high as 54℃ to 67℃. Water potential(low water content) and physiological aspects of the bacteria(stationary phase) could explain only in part of the prolonged survival of *E. coli* in compost. Therefore, it may be considered that there are other factors, such as resuscitation from VBNC state(Grimes, 1996; McDougald, 1998), which cause the survival of *E. coli* in compost.

3 Conclusions

According to the results of compost samples, coliform bacteria and salmonella exist in high concentration in raw materials, and most of them are destructed in composting, however some pathogenic bacteria survive in compost products, and even in uncompleted compost though the temperature of composting process is high(54—67℃). In the heat sensitivity experiments, *E. coli* in compost of high moisture content is more sensitive than in that of lower moisture content; in logarithmic phase the bacterium is more sensitive than that in stationary phase; and *E. coli* K12 is more sensitive than *E. coli* O157. Based on the *D* value, the lethal time of *E. coli* K12 and O157 will be 16.3 and 28.8 min, respectively, at 60℃ in compost of 40% moisture content. It is likely to say that there are also other important factors for the prolonged survival of *E. coli* in compost.

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