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Fate of *Escherichia coli* O157: H7 in agricultural soils amended with different organic fertilizers

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HIGHLIGHTS

- *Escherichia coli* O157:H7 survival time (t_d) prolonged as a result of organic fertilizers application, especially in acidic soils.
- Shorter t_d values were observed in soils amended with chicken manure and the longest in soils amended with pig manure.
- The t_d values are longer in original soils with higher pH and lower free Fe_2O_3.
- Electrical conductivity played a more important role in regulating *E. coli* O157:H7 survival in fertilizer-amended soils.

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ABSTRACT

Five organic fertilizers (vermicompost, pig manure, chicken manure, peat and oil residue) were applied to agricultural soils to study their effects on the survival of *Escherichia coli* O157:H7 (*E. coli* O157:H7). Results showed that *E. coli* O157:H7 survival changed greatly after organic fertilizers application, with shorter t_d values (survival time needed to reach the detection limit of 100 CFU g^{-1}) (12.57 ± 6.57 days) in soils amended with chicken manure and the longest (25.65 ± 7.12 days) in soils amended with pig manure. Soil pH, EC and free Fe/Al (hydro) oxides were significant explanatory factors for *E. coli* O157:H7 survival in the original soils. Soil constituents (minerals and organic matter) and changes in their surface charges with pH increased the effect of soil pH on *E. coli* O157:H7 survival. However, electrical conductivity played a more important role in regulating *E. coli* O157:H7 survival in fertilizer-amended soils. This study highlighted the importance of choosing appropriate organic fertilizers in the preharvest environment to reduce food-borne bacterial contamination.

1. Introduction

During the past decade, outbreaks of food-borne illnesses associated with the consumption of contaminated food produce continue to be a public health problem. Fresh produce can be contaminated with various human pathogens including bacteria, protozoa and viruses. Among the bacterial pathogens, numerous outbreaks are caused by *Escherichia coli* O157:H7 (*E. coli* O157:H7), which is dangerous because of its low infective dose (as few as 10 cells), high pathogenicity and ability to survive under frozen conditions [1]. Moreover, the virulence genes were found to be transferable to non-pathogenic *E. coli* strains [2]. In China, the first *E. coli* O157:H7 strain was isolated from hemorrhagic colitis patients in Xuzhou city, Jiangsu Province in 1986. No recognized outbreak has yet been reported. But several sporadic cases of *E. coli* O157:H7 infections have been detected in many different provinces [3,4].

Raw fruits and vegetables, especially fresh-cut leafy greens, are increasingly being recognized as the foremost transmitting vehicles of *E. coli* O157:H7 [5]. Although, fresh produce can be contaminated at any point along the farm-to-consumption handling chain, the field application of raw manure or contaminated irrigation water in the primary production phase is a principal route of *E. coli* O157:H7 contamination [6–8]. Hence, it is critical to prevent fresh produce from preharvest contamination in order to achieve the delivery of microbiologically safe produce to consumers [5]. Knowledge of the behavior of *E. coli* O157:H7 in plants growing in substrates like soil is important to develop strategies for minimizing contamination.

Depending on the soil properties and environmental factors, the reported survival times of *E. coli* O157:H7 in soil ranges from several days to more than 6 months [6–10]. The availability of resources,

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Table 1
Chemical characteristics of five types of organic fertilizers.

Code	Fertilizer		EC	TN	OC	Fe_d	Al_d		NH_4^+–N	NO_3^--N	WSOC
		pH	mc/cm	g/kg				C/N	g/kg		
1	Vermicompost	7.7	1.6	18.8	309.7	1.7	0.6	16.5	3.11	0.09	5.8
2	Peat	7.9	22.4	60.0	509.7	1.9	2.8	8.5	2.87	0.05	4.1
3	Chicken manure	8.0	40.3	18.7	415.8	7.7	6.8	22.2	2.59	0.11	4.0
4	Pig manure	7.8	4.1	21.0	549.1	1.2	0.9	26.1	4.42	0.17	16.3
5	Oil residue	5.1	0.1	48.3	453.6	2.4	3.1	9.6	2.73	0.08	12.8

EC, electrical conductivity; TN, total nitrogen; OC, organic carbon; Fe_d, free Fe_2O_3; Al_d, free Al_2O_3; C/N, the ratio of organic carbon to total nitrogen; WSOC, water soluble organic carbon.

such as carbon substrates, is probably a principal factor that affects the persistence of *E. coli* O157:H7 in natural environments, such as soil and water [6]. Utilization of organic materials (e.g., farmyard manure and slurry) is the most economic and practical option for improving soil quality while also providing additional sources of nutrients for growing plants [8]. Approximately 10^{11} ton of agricultural animal manure are produced globally each year and routinely applied to the soil as a crop fertilizer [11]. However, the application of organic materials could increase the incidence of disease caused by soil-borne fungal pathogens [12,13]. Likewise, the application of organic fertilizer may also influence the survival capacity of *E. coli* O157:H7 in soils [8,14]. For instance, Unc and Goss [14] reported that *E. coli* survived longer in soils amended with swine manure. Currently, most reports are concerned with animal manures and/or slurries [6,8,14]. Fertilizers such as peat and oil residue, are main organic fertilizers used worldwide. Little is known about the effect of other kinds of organic fertilizers on the survival of human enteric pathogens in agricultural soils.

In the present study, soils from vegetable fields with various physico-chemical properties were collected to study how *E. coli* O157:H7 survival is affected by the application of different organic fertilizers. More specifically, the goals were to: (i) investigate the factors regulating *E. coli* O157:H7 survival in soils, (ii) identify the effect of different fertilizer-amended soil characteristics which are responsible for differences in *E. coli* O157:H7 survival, and (iii) assess the possible risk of *E. coli* O157:H7 contamination when soil is amended with organic fertilizer.

2. Materials and methods

2.1. Organic fertilizers and soils

Five types of organic fertilizers (vermicompost, pig manure, chicken manure, peat and oil residue) which have various properties, were used in this study (Table 1). Pig manure was obtained from a commercial pig farm and air-dried. The other organic fertilizers were produced commercially by the same company. Four soils (P, H, N and E) of surface horizon (0–20 cm) were collected from vegetable fields in eastern China (P, 29.036°N, 119.452°E, Plinthudult; H, 30.349°N, 120.202°E, Hapludult; N, 28.790°N, 121.524°E, Endoaquept; E, 36.466°N, 115.972°E, Eutrochrepts). Triplicate plots were chosen randomly in each vegetable field. The soil sample from each plot was a composite of 5 individual soil cores taken at 5 m intervals. Samples were transported to the laboratory using coolers filled with ice, hand-picked to remove plant parts, stones, and earthworms, sieved (<2 mm), and stored at 4 °C before use. The selected soil physico-chemical properties are listed in Table 2.

Table 2
The selected properties of the test soils[a].

Soil code		EC	TN	OC	Fe_d	Al_d		NH_4^+-N	NO_3^--N	WSOC
	pH	ms/cm	g/kg				C/N	mg/kg		
P	4.6	0.21	1.9	34.4	11.2	3.53	18.1	2.29	5.93	56.4
P1	6.1	0.22	2.4	43.6	9.0	3.28	18.0	131.1	32.8	116.4
P2	6.0	0.23	4.2	51.6	11.2	3.76	12.4	511.5	24.1	594.5
P3	6.0	0.68	2.5	48.2	10.9	4.33	19.2	253.3	21.7	99.1
P4	6.3	0.32	2.6	54.2	11.0	3.55	21.0	39.5	33.1	254.0
P5	5.8	3.46	3.8	49.7	9.8	3.35	13.3	828.1	25.3	1422.8
H	5.3	0.52	1.7	18.6	24.5	13.09	10.9	6.47	32.9	142.8
H1	6.6	0.22	2.4	28.6	18.4	9.33	12.1	77.9	59.0	102.9
H2	6.4	2.10	3.8	36.7	17.9	9.27	9.6	348.7	761.5	537.1
H3	6.3	1.78	2.3	33.2	12.2	7.89	14.2	280.3	222.7	76.1
H4	6.9	0.34	2.4	38.8	15.0	8.91	16.1	48.3	78.8	253.7
H5	6.1	3.23	3.3	34.3	20.1	9.99	10.3	634.4	642.8	1765.0
N	5.6	0.53	2.6	27.4	4.5	1.36	10.7	7.1	36.1	157.2
N1	6.5	0.58	3.2	37.9	3.3	1.12	12.0	98.2	53.3	94.3
N2	6.5	2.33	4.7	44.2	2.7	1.60	9.5	545.9	58.5	611.7
N3	6.4	2.03	3.1	42.7	3.5	1.71	13.6	402.3	56.9	103.0
N4	6.7	0.58	3.2	47.1	2.7	1.44	14.6	92.6	57.9	198.5
N5	6.0	3.00	4.3	43.4	2.7	1.55	10.2	689.9	58.4	1363.7
E	8.2	0.13	1.7	20.2	3.1	0.88	12.0	2.7	81.0	68.1
E1	8.4	0.41	2.3	30.8	3.1	0.88	13.7	85.8	42.6	66.4
E2	8.3	2.64	3.9	39.2	2.9	0.99	10.1	231.9	32.0	625.8
E3	8.2	2.14	2.3	34.5	3.3	1.28	15.0	363.5	39.1	58.8
E4	8.3	0.60	2.4	40.6	3.8	1.15	16.6	41.5	52.9	166.1
E5	7.6	3.54	3.4	35.7	1.7	0.99	10.6	717.4	33.1	1787.2

[a] The abbreviations of physico-chemical properties are the same as shown in Table 1; P, H, N, E represent different kinds of agricultural soils (Plinthudult, Hapludult, Endoaquept, Eutrochrepts) in China, respectively. The soils with organic fertilizers were suffixed with the organic fertilizers codes of 1–5 as mentioned in Table 1.