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Persistence of *Escherichia coli* O157 and non-O157 strains in agricultural soils

Jincai Ma [a,b], A. Mark Ibekwe [a,*], David E. Crowley [b], Ching-Hong Yang [c]

[a] USDA-ARS U. S. Salinity Laboratory, Riverside, CA 92507, United States
[b] Department of Environmental Sciences, University of California, Riverside, CA 92521, United States
[c] Department of Biological Sciences, University of Wisconsin, Milwaukee, WI 53211, United States

HIGHLIGHTS

- *E. coli* O157:H7 and non-O157 persisted differently in soils.
- EC, pH, silt, clay content, and T-N influenced the persistence of *E. coli* O157.
- The effects of environmental factors on Non-O157 were both strain and soil specific.
- *E. coli* O103:H2 survived two to three times longer than *E. coli* O157:H7.

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ABSTRACT

Shiga toxin producing *Escherichia coli* O157 and non-O157 serogroups are known to cause serious diseases in human. However, research on the persistence of *E. coli* non-O157 serogroups in preharvest environment is limited. In the current study, we compared the survival behavior of *E. coli* O157 to that of non-O157 *E. coli* strains in agricultural soils collected from three major fresh produce growing areas of California (CA) and Arizona (AZ). Results showed that the nonpathogenic *E. coli* O157:H7 4554 survived longer than the pathogenic *E. coli* O157:H7 EDL933 in Imperial Valley CA and Yuma AZ, but not in soils from the Salinas area. However, *E. coli* O157:NM was found to persist significantly longer than *E. coli* O157:H7 EDL933 in all soil tested from the three regions. Furthermore, two non-O157 (*E. coli* O26:H21 and *E. coli* O103:H2) survived significantly longer than *E. coli* O157:H7 EDL933 in all soils tested. Pearson correlation analysis showed that survival of the *E. coli* strains was affected by different environmental factors. Our data suggest that survival of *E. coli* O157 and non-O157 may be strain and soil specific, and therefore, care must be taken in data interpretation with respect to survival of this pathogen in different soils.

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1. Introduction

Shiga toxin producing *Escherichia coli* (STEC) are pathogens that are known to cause many outbreaks of foodborne infections in human (Kaper et al., 2004). Hundreds of STEC strains have been isolated from healthy animals, infected humans, and contaminated foods. Although *E. coli* O157:H7 is reported to be the most predominant STEC serotype in the United States, more than 200 non-O157 STEC serotypes have been identified in animals or foods (Karch et al., 2005). Recent epidemiological studies have recognized additional non-O157 serotypes, including O26, O45, O91, O103, O104, O111, O113, O121, and O145, among STEC strains that were linked to severe human disease in the United States, Europe and parts of Latin America (Bettelheim, 2007; Beutin and Martin, 2012; Brooks et al., 2005; Caprioli et al., 2005; Mathusa et al., 2010). Approximately, 60 of these serotypes have been incriminated in human diseases. Common non-O157 STEC causing diseases in the United States include O111, O26, O121, and O103 (Johnson et al., 1996). The clinical symptoms caused by non-O157 STEC infections may be similar to O157 STEC induced diseases. The typical clinical manifestation caused by STEC ranges from mild bloody diarrhea (hemorrhagic colitis, HC) to fatal hemolytic uremic syndrome (HUS) (Johnson et al., 2006). Data of a previous survey revealed that there is a significant burden of non-O157 STEC associated disease in the United States. Epidemiology data from Canada, Europe, Argentina, and Australia suggest that infections caused by non-O157STEC might be as prevalent as, or even more than

* Corresponding author at: USDA-ARS-U. S. Salinity Laboratory, 450 W. Big Springs Rd, Riverside, CA 92507, United States. Tel.: +1 951 369 4828; fax: +1 951 342 4964.
E-mail address: Mark.Ibekwe@ars.usda.gov (A. Mark Ibekwe).

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those caused by STEC O157 strains (Fey et al., 2000). Therefore, there is an increasing concern over the risk of non-O157 STEC strains to the food industry, regulatory officials, and the general public (van Elsas et al., 2011).

STEC mainly originate from ruminants with cattle being the super shedders (Chase-Topping et al., 2008). Consumption of under-cooked beef is the major pathway for STEC outbreak (Besser et al., 1999). In recent years, growing evidence has shown that fresh produce is an important vector for STEC transmission (Berger et al., 2010; Tyler and Triplett, 2008). Since 1995, 9 of these outbreaks out of 22 outbreaks of *E. coli* O157:H7 infections were traced to, or near the major fresh produce growing area in Salinas, California (Cooley et al., 2007). In 2006, a large outbreak in the United States was found to be associated with bagged fresh spinach, and the outbreak resulted in 199 infections, including 31 HUS and 3 deaths (Cooley et al., 2007). On the other hand, STEC strains belonging to non-O157 were incriminated in large-scale outbreaks and sporadic infections via consumption of fresh produce (CDC, 2010). Recently, a non-O157 STEC, *E. coli* O104:H2, was believed to be the cause of a large outbreak of food borne infection in Germany (Beutin and Martin, 2012). During this outbreak, more than 4000 hospitalizations including 50 deaths were reported (Laing et al., 2012).

It is quite clear that both O157 and non-O157 STEC can cause serious diseases in human via consumption of fresh produce (Cooley et al., 2007; van Elsas et al., 2011), therefore, intensive studies are needed toward a better understanding of fate of both O157 and non-O157 STEC on and near fresh produce growing farms. These pathogens could enter into the environment via different pathways. Water, sediments, and soil are major secondary reservoirs of STEC in the environment. Once STEC is in the soils, especially in agricultural soils, they might persist for days and even months, thus increasing the chances that they could enter into the food chain and constitute a major public health risk (Jones et al., 1999). Agricultural soils might be contaminated with STEC via manure application, runoff from animal farms, feces by wild animals, and possibly deposition of dust containing STEC (Cooley et al., 2007).

Recently research has shown that plant might be a potential habitat for food-borne pathogens, and more importantly fresh produce is a major vehicle in the transmission of STEC (Berger et al., 2010; Tyler and Triplett, 2008). Foodborne outbreaks due to contaminated fresh produce are a growing burden (Lynch et al., 2009). Fresh produce might get contaminated by STEC during the pre-harvest or post-harvest process. The STEC source in preharvest stage might include water (irrigation water and natural precipitation), soils (organic fertilizer), and air (particulate deposition). However, more research is needed on the soil–plant–microbe interaction to investigate the persistence of STEC in soils used for growing fresh produce. The longer the pathogens survive in the soils, the higher the chances of those pathogens contaminating fresh produce. It was also reported that STEC might proliferate in plant, and plant lesions can promote the multiplication of *E. coli* O157 on postharvest fresh produce (Brandl, 2008; Solomon et al., 2002), thus increasing the risk associated with those pathogens. Currently, most of the studies focus on *E. coli* O157. Persistence of *E. coli* O157 in manure-amended soil (Franz et al., 2008), and natural soils (Ma et al., 2011), are well documented. However, survival data on O157 and non-O157 serotypes in agricultural soils used for fresh produce production is limited.

In the current study, we chose three O157 strains (*E. coli* O157:H7 EDL933, *E. coli* O157:NM, *E. coli* O157:H7 4554), and three non-O157 strains (*E. coli* O26:H11, *E. coli* O91:H21, and *E. coli* O103:H2), to investigate their survival behavior in agricultural soil from major leafy green growing areas of Arizona and California. The results obtained offer new insights into the survival behavior of *E. coli* O157 and non-O157 strains in typical fresh produce growing agricultural soils, and provide additional data that may help in the development of managerial strategies to control on-farm preharvest contamination.

2. Materials and methods

2.1. Bacterial strains

The *E. coli* strains used in this study are described in Table 1. In order to facilitate the enumeration of these strains on selective media, the *E. coli* strains were tagged with nalidixic acid and rifampicin resistance, and their growth profiles in M9 medium was found to be identical to that of the non-tagged wild-type strains (data not shown). All the nalidixic acid and rifampicin tagged strains and their corresponding wild type strains were separately stored under −80 °C on cryoprotective beads in MicroBank microbial storage tubes (Pro-Lab Diagnostics, Ontario, Canada).

2.2. Growth curve in M9 medium

The strains were inoculated into 100 ml of M9 medium, and grew under 37 °C with a rotation rate of 250 rpm. The optical density at 600 nm (OD_{600nm}) was monitored using a VIS–UV spectrophotometer (Pharmacia Biotech Inc. NJ). The OD_{600nm} was plotted against incubation time, and the apparent growth rate (k, h^{-1}) was calculated using the following equation,

$$k = (OD_2 - OD_1)/(t_2 - t_1)$$

where OD_1, OD_2 are the optical density measured at time t_1 and time t_2, respectively, and k is the apparent growth rate (h^{-1}).

2.3. Multiplex PCR targeting virulence genes

Multiplex PCR was performed on both *E. coli* O157 and non-O157 strains to investigate the presence of the virulence genes including *hylA*, stx_1, stx_2, and *eae* genes. PCR was performed using Ready-to-Go PCR beads with the four primer sets targeting *hylA*, stx_1, stx_2, and *eae* gene, respectively (Paton and Paton, 1998). Thermocycler protocol included an initial denaturation at 95 °C for 10 min, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 55 °C for 30 s, and extension at 72 °C for 40 s, and a final extension at 72 °C for 5 min. The PCR product was resolved by electrophoresis on a 1.0% agarose gel. The gel was then stained with ethidium bromide, visualized and photographed using a gel imaging system (Bio-Rad Lab., Hercules, CA).

Table 1
E. coli O157 and non-O157 strains.

Strain	Source	stx_1	stx_2	*eae*	*hylA*	Reference
E. coli O26:H11	Cow, Ontario, Canada	−	+	+	+	(Louie et al., 1998)
E. coli O103:H2	Cow, Ontario, Canada	+	+	+	+	(Louie et al., 1998)
E. coli O91:H21	Human, OH, USA	+	−	−	+	(Ito et al., 1990)
E. coli O157 NM	–, AL, USA	+	+	+	+	(Fields et al., 1997)
E. coli O157:H7 4554	cow, Japan	−	−	+	+	(Feng et al., 2001)
E. coli O157:H7 EDL933	human, USA	+	+	+	+	(Perna et al., 2001)

stx_1, a gene coding for Shiga toxin1, stx_2, a gene coding for Shiga toxin2, *eae*, a gene coding for intimin, and *hylA*, a gene coding for hemolysin. " + " and "−" indicate a gene was identified and not identified in a given *E. coli* strain, respectively.

2.4. Pulse field gel electrophoresis analysis

PFGE was conducted to analyze genetic similarities between *E. coli* O157 and non-O157 strains. Genomic DNA fingerprints of the *E. coli* strains were determined using pulsed field gel electrophoresis (PFGE) according to the standard protocol developed by PulseNet, Center for Disease Control, USA (Ribot et al., 2006). Briefly, genomic DNA for PFGE was prepared by lysing *E. coli* O157 cells that were encased in 1% Sea Kem Gold agarose plugs. After multiple washes, the DNA embedded in the agarose plugs was digested with *Xba*I at 37 °C for at least 3 h, and the resultant fragments were separated by electrophoresis in 1.0 × Tris-Borate-EDTA buffer at 14 °C for 16 h on a 1% Sea Kem Gold agarose gel on a CHEF III Mapper (Bio-Rad Lab., Hercules, CA, USA) with initial switch time of 2.16 s and final switch time of 35.07 s, respectively. The low range PFG marker (New England Biolabs, Ipswich, MA) was used as reference. The agarose gel was stained with ethidium bromide, destained in Milli-Q water twice, and the banding pattern was visualized and photographed using a gel imaging system. Comparison of digested profiles to identify restriction enzyme digestion pattern clusters was performed with the BioNumerics software, version 5.0 (Applied Maths, Austin, TX). Fingerprints were clustered using the Jaccard coefficient evaluated by the unweighted-pair group method.

2.5. Collection, characterization, and inoculation of soils samples

Soil samples (organically and conventionally managed) were collected from each of three major fresh produce growing areas, including Yuma, Arizona, Imperial Valley, California, and Salinas, California as previously described (Ma et al., 2012b). The typical fresh produce grown in these areas are spinach and lettuce. Soil samples were collected, sieved (2 mm), put into plastic bags, and stored at 4 °C in the dark until use. Soil properties characterized (Ma et al., 2012b) included clay, silt, and sand content, pH, electrical conductivity (EC), bulk density, water content, water-holding capacity (WHC), total organic carbon (OC), and total nitrogen (T-N) (Klute, 1996). Soil microbial biomass carbon (MBC) was extracted by the chloroform-fumigation-extraction method (Vance et al., 1987). The texture and chemistry of the soils were summarized in Table 2.

Cells from stock cultures were streaked on LB agar (without antibiotics), and incubated at 37 °C overnight. Single colonies were picked and restreaked onto LB agar with appropriate antibiotics. Single colonies were streaked onto SMAC (sorbitol MacConkey) agar supplemented with BCIG (5-bromo-4-chloro-3-indoxyl-β-D-glucuronide) (Lab M, Lancashire, UK). The isolated colonies were inoculated into 100 ml LB broth with appropriate antibiotics, and incubated at 37 °C for about 16 h. Stationary phase cells were used because in the natural environment, the majority of bacteria exist in this condition (Kolter et al., 1993). The overnight cultures were harvested by centrifugation at 4 °C, washed three times with phosphate buffer (10 mM, pH 7.2), resuspended in sterile deionized water, and inoculated into soil samples. The washed step was essential to remove the nutrient, typically organic carbon from the LB broth, since *E. coli* O157 is able to grow at low carbon concentrations in freshwater (Vital et al., 2010). Cells were added into soils to a final density of about 0.5×10^7 CFU per gram soil (g dw^{-1}). Briefly, the cell suspension was thoroughly mixed with soil in a plastic bag and 500 g of the inoculated soil was transferred to a top perforated plastic bag for air exchange. The same amount of non-inoculated soil was put into another plastic bag, which was used as uninoculated control with deionized water being added instead of cell suspension. The control experiments were used for the determination of moisture loss and check of potential contamination. The plastic bags were weighed and incubated at room temperature (20 ± 2 °C) in the darkness. Triplicate bags were prepared for control and inoculated soil samples. Moisture content of the soil samples was adjusted to 60% WHC, and water concentration was maintained the same during the course of an experiment by adding deionized water weekly to obtain the original weight. Antibiotics were added into the agar media at the following concentrations, rifampicin (Rif), 100 μg ml^{-1}; and nalidixic acid (Nal), 25 μg ml^{-1}.

2.6. Sampling and enumeration

The inoculated soils were sampled periodically to determine the survival of the *E. coli* strains over time. At each point, two samples (1.0 g) of each replicate were removed from the middle of the soil sample and put into pre-weighed dilution tubes. The tubes containing soil samples were weighted to calculate the exact size of soil sample. A 5.0 ml of 0.1% peptone buffer (Lab M, Lancashire, UK) was added to the test tube containing the soil sample, and the soil was thoroughly mixed with the buffer by inverting the tube several times and then vortexed for 2 × 20 s. The resulting soil paste (cell suspension) was then subjected to 10-fold serial dilutions. 50 μl of the two highest dilutions was plated in duplicate on SMAC/BCIG agar with appropriate antibiotics for enumeration. The inoculated SMAC agar plates were incubated at 37 °C for 16 h, and the results expressed as log colony-forming units per gram dry weight (CFU g dw^{-1}). The detection limit of the plating method was approximately 100 CFU g dw^{-1}.

2.7. Survival data modeling

Survival of *E. coli* strains was modeled by fitting the experimental data to the Weibull survival model proposed by Mafart et al. (2002) using GInaFiT version 1.5 (Geeraerd et al., 2005). The Weibull survival model was constructed based on the hypothesis that the deactivation kinetics of the *E. coli* population follows a Weibull distribution. The size of the surviving population can be calculated using Eq. (1),

$$\log(N_t) = \log(N_0) - (t/\delta)^p. \quad (1)$$

Where N is the number of survivors, N_0 is the inoculum size; t is the time; p is the shape parameter, when $p > 1$ a convex curve is observed; when $p < 1$ a concave curve is observed, when $p = 1$a linear curve is observed. The scale parameter, δ, represents the time needed for first decimal reduction. The strong correlation between the scale (δ) and the shape (p) parameters makes the double Weibull model to fit most

Table 2
Soil properties.

Soil ID	soil texture	pH	EC (dS m^{-1})	WHC (%)	Sand (%)	Clay (%)	BD (g cm^3)	T-N (%)	OC (%)	MBC (mg kg^{-1})
AZ1	Clay	7.9	1.21	49.5	17.5	44.1	1.25	0.067	1.93	540.8
AZ2	Clay	7.9	2.06	47.7	17.5	43.3	1.25	0.065	1.79	505.3
IM1	Clay loam	7.7	1.92	39.9	31.2	32.9	1.32	0.089	2.04	277.8
IM2	Clay loam	7.7	1.22	37.0	22.5	35.5	1.29	0.049	2.08	364.3
SA1	Sandy loam	6.9	0.37	25.8	74.4	10.0	1.58	0.140	1.58	197.6
SA2	Sandy loam	7.4	0.64	21.4	78.1	8.8	1.60	0.128	1.28	163.7

AZ (Yuma, Arizona), IM (Imperial Valley, California, and SA (Salinas, California) presents the locations where the soil sample were collected; EC, electrical conductivity or salinity; WHC, water holding capacity; BD, bulk density; T-N, total nitrogen; OC, organic carbon; WSOC, water soluble organic carbon in soil water extract (soil:water, 1:1); and MBC, microbial biomass carbon. soil #1 and soil #2 were organic and conventional soils, respectively.

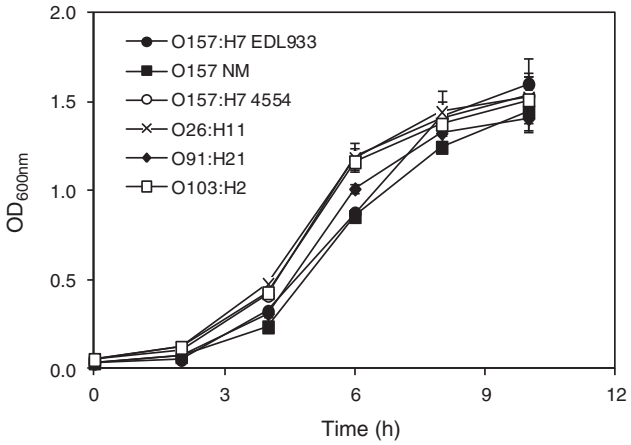

Fig. 1. Growth curves of *E. coli* O157 and non-O157 strain in M9 medium.

of the shapes of deactivation curves. Previous study proved that the double Weibull model can successfully describe a biphasic shape with nonlinear decrease, which cannot be described by other survival models. A very important and useful parameter, *ttd* (time needed to reach detection limit, 100 CFU g dw^{-1}) can also be calculated when using GInaFiT to fit the experimental survival data.

2.8. Statistical analysis

Analysis of variance (ANOVA) was performed to investigate the differences in growth in M9 medium, and Pearson correlation between *ttd* and soil properties was performed using SPSS 16.0 software package (Chicago, IL). Clustering analysis of $\log_{10}(X + 1)$ transformed survival parameters (*ttd*, δ, and p) was performed using CLUSTER3.0 (http://rana.stanford.edu), and the heat map was visualized using JAVA TREEVIEW (http://rana.stanford.edu/). Brighter red coloring indicates larger the parameters.

3. Results

3.1. Multiplex PCR

Multiplex PCR results (Table 1) showed that *E. coli* O157:H7 EDL933, *E. coli* O157 NM, and *E. coli* O103:H2, harbor all four virulence genes, from top to bottom, enterohemolysin (*ehxA*), intimin (*eae*), Shiga toxin 1 (stx_1), and Shiga toxin 2 (stx_2). *E. coli* O157:H7 4554 is a shiga-toxin negative strain (non-pathogenic), which does not have either stx_1 or stx_2 genes. *E. coli* O26:H11 confers stx_2, *ehxA*, and *eae*, but not stx_1 gene, while *E. coli* O91:H21 is positive for stx_1 and *ehxA* but not *eae* and stx_2 genes in its genome.

3.2. Growth in M9 medium

The growth experiment demonstrated that all the *E. coli* strains, both O157 and non-O157 strains, displayed an identical growth profile when inoculated into M9 medium (Fig. 1). The apparent growth rate (k, h^{-1}) for *E. coli* O157:H7, *E. coli* O157:NM, *E. coli* O157:H7 4554, *E. coli* O26:H11, *E. coli* O91:H21, and *E. coli* O103:H2 was calculated as 0.27 ± 0.03, 0.25 ± 0.03, 0.25 ± 0.03, 0.24 ± 0.03, 0.24 ± 0.02, respectively.

3.3. PFGE profiles

To detect genetic similarities among STEC strains, we performed PFGE on the six *E. coli* isolates of both human and animal origins. Isolates were considered indistinguishable if they had the same number and size of bands in a PFGE fingerprint pattern. Isolates were considered to be closely related if their PFGE pattern differed by changes consistent with a single genetic event. Such strains typically do not have differences in more than three bands. The six *E. coli* strains were clustered into two groups (Fig. 2), with group 1 dominated by the three *E. coli* non-O157 strains, and the other cluster contained the three O157 strains. It was also found that the stx_1 and stx_2 negative strain *E. coli* O157:H7 strain 4554 shares a 72% of genomic information with *E. coli* O157:H7 EDL933. None of the strains had the same PFGE patterns.

3.4. Survival patterns of E. coli O157 and non-O157 strains

Overall, the three *E. coli* O157 strains survived longer ($p < 0.05$) in organic soil than in conventional soils from Imperial Valley. Similarly, *E. coli* O157:NM and *E. coli* O26:H21 lasted longer ($p < 0.05$) in organic soils than in conventional soils from Salinas. When inoculated in soils from Yuma, Arizona, *E. coli* O26:H21 and *E. coli* O103:H2 were more persistence ($p < 0.05$) in organic soils than in conventional soils (Fig. 3). It was found that *E. coli* O91:H21 survived the shortest, and the longest survival was observed for O103:H2 in all soils tested (Fig. 3).

Survival of *E. coli* O157:H7 EDL933 in soils from Yuma, Imperial Valley, and Salinas Valley was comparable to the survival data from our previous study, where it lasted up to 31 days (Ma et al., 2012a,b). In the current study, *ttd*s of *E. coli* O26:H11 and *E. coli* O103:H2 were relatively longer than the other *E. coli* strains. On average, *ttd*s for *E. coli* O26:H11 were about twice the values of *E. coli* O157:H7 EDL933 when inoculated into soils regardless of their collection areas. *E. coli* O103:

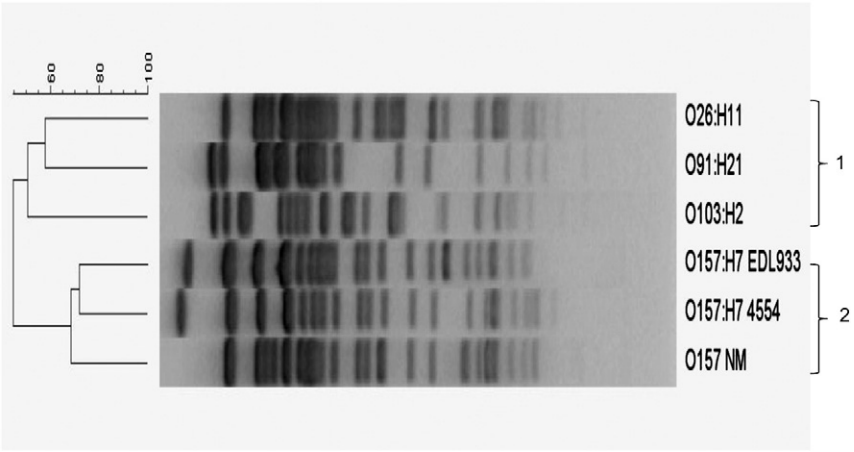

Fig. 2. Dendrogram comparing strain relationship of *E. coli* O157 and non-O157 strains. The cluster tree was constructed using molecular analysis software (Bio-Rad), from comparison of *Xba*I-digested pulse field gel electrophoresis (PFGE) patterns.

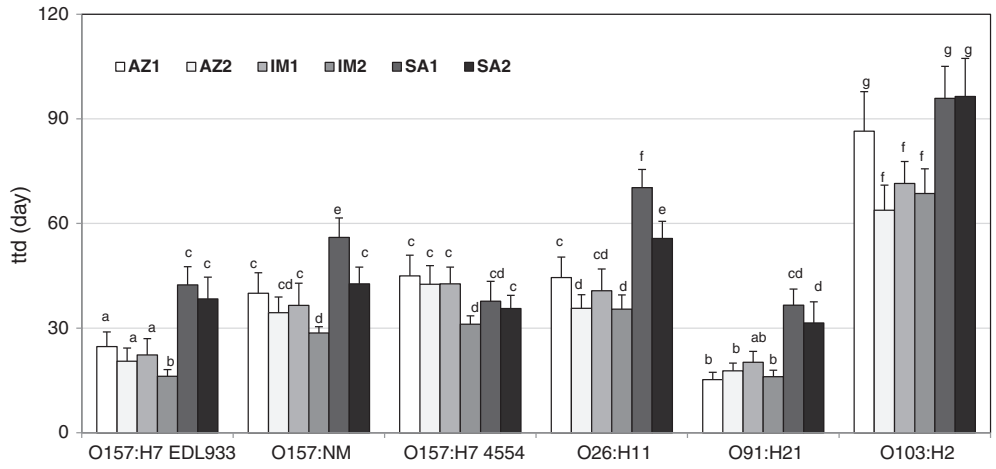

Fig. 3. Survival of *E. coli* O157:H7 EDL933, *E. coli* O157:NM, *E. coli* O157:H7 45554, *E. coli* O26:H11, *E. coli* O91:H21, and *E. coli* O103:H12 in organic soil (#1) and conventional soil (#2) from Yuma, Arizona (AZ), Imperial Valley, California (IM), and Salinas, California (SA). Error bars represent the standard deviation of the triplicate measurements of *ttd* (time to detection limit, 100 CFU g^{-1}). Bars labeled with different letters are significantly different ($n = 3$, $p < 0.05$).

H2 survived the longest in all soils tested, with its *ttd*s being 2.8, 3.6 and 2.4 times of those for *E. coli* O157:H7 EDL933 in soils from Arizona, Imperial Valley, and Salinas, respectively (Table 3).

The calculated δ values showed that in most cases the first log reduction time in cells population occurred within one week (Table 3). However, for *E. coli* O103:H2, it took about two weeks (16.7 and 17.8 days, respectively) to reach the first decimal reduction in cell population when it was inoculated into soils from Yuma and Imperial Valley, and δ values were nearly 2 months when it was inoculated in soils from Salinas (Table 3). For the shape parameters, p, most of the p values were below 1, indicating concave survival curves. Interestingly, the p values for *E. coli* O103:H2 were greater than 1, especially for the cells inoculated into the soils from Salinas where its p values were close to 3 ($p = 2.73$), indicating a convex shape of its corresponding survival curves.

As evidenced by the δ and p values shown in Table 3, the survival curves of *E. coli* cells inoculated in soils from Yuma and Imperial Valleys showed a concave shape with a relatively fast initial decline followed by a slower reduction phase until detection limit (100 CFU g^{-1}) was reached. In contrast, the survival patterns for *E. coli* O103:H2 in soils from Salinas displayed convex curves, and the population size stayed the same for almost two months during the slower reduction phase until the detection limit was obtained (data not shown).

Furthermore, heatmap (Fig. 4) showed that the survival profiles of *E. coli* strains were clustered into two groups, one cluster contained only one strain, *E. coli* O103:H2, while the rest of the five *E. coli* strains fell into the other group based on *ttd*, δ, and p. Additionally, it was found that the survival profiles of *E. coli* O157:H7 EDL933 and *E. coli* O91:H21 were about the same. Survival patterns of *E. coli* O157:NM and *E. coli* O26:H11 shared a lot of similarities. Further linear regression analysis (Fig. 5) showed that the overall *ttd* of *E. coli* strains was positively correlated with the first decimal reduction time (δ) and the shape parameter (p).

3.5. Pearson correlation

It was found that EC, pH, silt, clay content, and T-N significantly influence the persistence of *E. coli* O157 EDL933 in soils with T-N exerting a positive influence, while the other factors exerted negative effects (Table 4). T-N was a major factor controlling the increased in persistence of *E. coli* O157:NM. None of the environmental factors examined in this study had any significant effect on the survival of non-pathogenic *E. coli* O157:H7 4554. For the non-O157 strains, the effects of the different environmental factors were both strain and soil specific in most cases. pH played a major role in the survival of *E. coli* O26:H11 with higher pH resulting in greater persistence, and lower EC also resulted in significant increase in persistence of this strain. Other factors (Table 4) did not have any significant effect on the survival of this strain. For the survival of *E. coli* O103:H2, EC and silt contents were important factors in the determination of its survival in soils. OC was not significantly correlated with any *ttd* of *E. coli* strains. Overall, EC and silt content negatively affected survival, while T-N positively affects the survival of *E. coli* in soils. Other soil properties either positively or negatively correlated with the survival of strains tested in the soils.

Table 3
Averaged survival parameters of *E. coli* strains in soils from Yuma, Arizona (AZ), Imperial Valley, CA (IM), and Salinas Valley, CA (SA). Survival parameter *ttd* (time needed to reach detection limit, 100 CFU g^{-1}), was shown in means ± standard deviation of survival experiments in two soils from each area.

	E. coli O157:H7 *EDL933*	*E. coli* O157:NM	*E. coli* O157:H7 *4554*	*E. coli* O26:H11	*E. coli* O91:H21	*E. coli* O103:H2
ttd (day)						
AZ	23.6 ± 2.40	38.7 ± 2.40	43.8 ± 1.70	39.6 ± 2.69	16.5 ± 1.70	66.7 ± 4.03
IM	19.2 ± 4.17	33.6 ± 4.17	36.9 ± 3.96	38.1 ± 3.75	18.1 ± 2.97	70.1 ± 2.05
SA	40.4 ± 3.75	49.3 ± 3.75	36.6 ± 1.48	63.0 ± 3.96	34.0 ± 3.68	98.2 ± 1.06
δ (day)						
AZ	5.96 ± 0.59	4.19 ± 0.59	9.85 ± 2.51	6.00 ± 1.03	4.42 ± 1.52	16.7 ± 0.42
IM	2.51 ± 0.48	2.35 ± 0.48	7.26 ± 2.19	4.45 ± 0.72	2.65 ± 0.48	17.8 ± 1.98
SA	5.21 ± 0.24	6.92 ± 0.24	9.12 ± 0.55	13.3 ± 1.91	5.00 ± 1.30	56.7 ± 3.54
p						
AZ	0.91 ± 0.18	0.58 ± 0.18	0.84 ± 0.22	0.84 ± 0.06	0.98 ± 0.02	1.08 ± 0.13
IM	0.80 ± 0.08	0.55 ± 0.08	0.91 ± 0.08	0.74 ± 0.09	0.91 ± 0.00	1.09 ± 0.24
SA	0.79 ± 0.08	0.84 ± 0.08	1.18 ± 0.07	1.09 ± 0.21	0.88 ± 0.06	2.73 ± 0.27

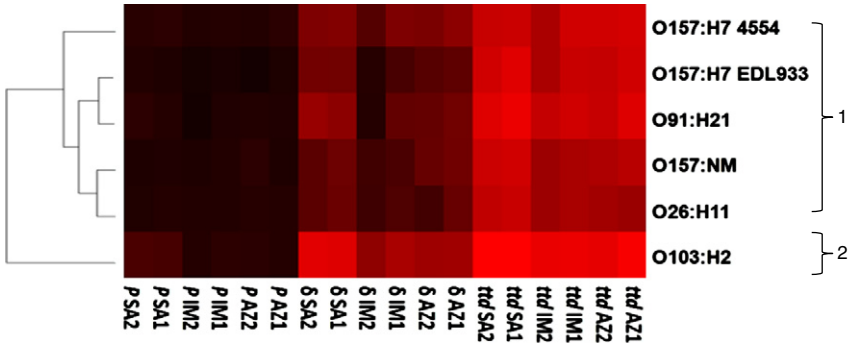

Fig. 4. Clustering analysis of survival profiles of survival of *E. coli* O157:H7 EDL933, *E. coli* O157:NM, *E. coli* O157:H7 45554, *E. coli* O26:H11, *E. coli* O91:H21, and *E. coli* O103:H12 in soils based on survival parameters including time to reach the detection limit (100 CFU g^{-1}), (*ttd*), first decimal reduction time (δ), and shape parameter (p). Heat maps were generated using CLUSTER and visualized using TREEVIEW. Brighter red coloring indicates higher values for *ttd*, δ, and *p*. AZ, soils from Yuma, Arizona; IM, soils from Imperial Valley, California; SA, soils from Salinas, California. Soil #1 and soil #2 were organic and conventional soils, respectively.

4. Discussion

Most survival studies focused mainly on *E. coli* O157:H7 strains, while survival of non-O157 strains received less attention (Bolton et al., 2011; Fremaux et al., 2008). However, in Europe and the United States, the role of non-O157 STEC strains (e.g. O26:H11, O91:H21, O103:H2, and O145:H28) as causes of HC, HUS, and other gastrointestinal illnesses is being increasingly recognized (Johnson et al., 1996; Kappeli et al., 2011). According to published data, *E. coli* O157 can survive from several weeks to six months (Bolton et al., 1999; Ma et al., 2011; Semenov et al., 2008). All inoculated non-O157 serotypes survived longer in the sandy loam soil with survival time (*D*-values) ranging from 50.26 to 75.60 days, while in clay loam soils the range was 31.60–48.25 days (Bolton et al., 2011; Fremaux et al., 2008), both of which fell into the survival range obtained in the current study (16.5–98.2 days).

In this study, we found that except *E. coli* O157:H7 4554, the other five *E. coli* strains survived longer in soils near Salinas Valley than in soils from Yuma and Imperial Valley. This is in line with our previous report showing that *E. coli* O157:H7 EDL933 persisted longer when inoculated into soils from Salinas Valley area than soils from Yuma and Imperial Valley (Ma et al., 2012a). Salinas Valley area is one of the major fresh produce growing areas in the United States, and supplies about 30% of fresh produce during the summer growing season. Considering the relatively lower annual mean temperature and higher annual mean precipitation in the Salinas Valley, the soils from this area may favor the persistence of potential food pathogens more compared to the soils from other two areas. The reasons for longer survival in Salinas soils are still not fully understood, but possible reasons might be the lower level of salinity and higher assimilable organic carbon in those soils as reported recently (Ma et al., 2012a; Erikson et al., 2013). Obviously, additional research is needed toward a better understanding of the mechanisms involved in longer persistence of human pathogens in soils from Salinas Valley areas, CA.

Pearson correlation results revealed an interesting trend that survival of each strain was affected by different environmental factors, implying that different strains might possess distinct strategies to persist in a given soil, which might be related to their central genomes and ability to respond to various environmental stresses. It was also found that the cluster patterns based on PFGE is different from the one based on survival parameters, indicating that molecular genetics of *E. coli* strains is still poorly understood as it relates to their survival behavior in the soils. The potential mechanisms might include stress adaptation and nutrient metabolism (Franz et al., 2011). Some special mechanisms might play an important role in helping the STEC strain to survive longer in soils, which is still yet to be elucidated.

In the current study, it was found that the first log reduction time (δ) was significantly correlated with *ttd*. This is well in line with our previous report that showed δ was positively related with *ttd* of EDL933 in agricultural soils. Since δ is easier to obtain, it could be an alternative indicator of *E. coli* strains survival in soils (Ma et al., 2012a). Additionally, when all six strains were taken into account, the *ttd* was found to be positively correlated with the shape parameter (p), suggesting that a concave ($p < 1$) survival shape may correspond to shorter *ttd*, while a convex ($p > 1$) survival shape may correspond to a longer *ttd*.

It should be noted that in the current study only a limited number of STEC strains were used, and the data obtained might not yield a general

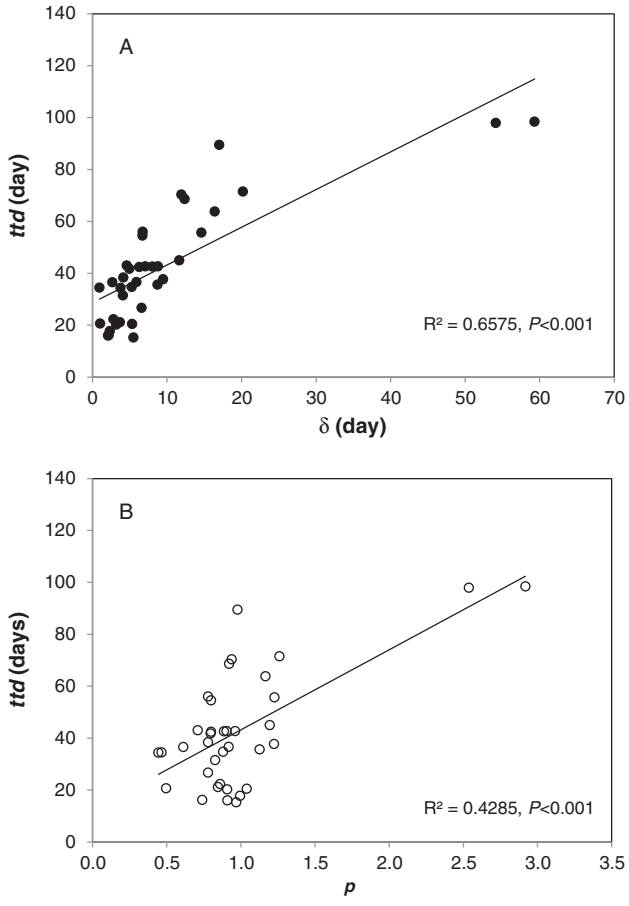

Fig. 5. Linear regression analysis of *ttd* of *E. coli* strains with the first decimal reduction time (δ) (A) and the shape parameter (p) (B). *ttd*, time needed to reach detection limit (100 CFU g^{-1}); δ, first decimal reduction parameter; and *p*, shape parameter. AZ, soils from Yuma, Arizona; IM, soils from Imperial Valley, California; SA, soils from Salinas, California. Soil #1 and soil #2 were organic and conventional soils, respectively.

Table 4
Pearson correlation between *ttd* (time to reach detection limit, 100 CFU g^{-1}) and soil properties.

Strains	EC	pH	WHC	Silt	Clay	T-N	OC	MBC
E. coli O157:H7 EDL933	−0.82*	−0.84*	−0.77	−0.92**	−0.83*	+0.87*	−0.77	−0.69
E. coli O157:NM	−0.30	−0.74	−0.51	−0.73	−0.57	+0.81*	−0.51	−0.45
E. coli O157:H7 4554	−0.57	+0.05	+0.19	−0.06	+0.22	+0.35	+0.06	+0.22
E. coli O26:H11	−0.82*	+0.82*	−0.62	−0.79	−0.72	+0.77	−0.58	−0.50
E. coli O91:H21	−0.76	−0.97**	−0.93**	−0.96**	−0.95**	+0.88**	+0.77	−0.91**
E. coli O103:H2	−0.88*	−0.71	−0.68	−0.83*	−0.76	+0.71	−0.72	−0.54

EC, electrical conductivity or salinity; WHC, water holding capacity; BD, bulk density; T-N, total nitrogen; OC, organic carbon; WSOC, water soluble organic carbon in soil water extract (soil:water, 1:1); MBC, microbial biomass carbon.
* Denotes significant at 0.05.
** Denotes significant at 0.01.

trend on the survival profiles of *E. coli* O157 and non-O157 strains in soils. However, the data in current study revealed the complicated interaction between STEC and soils (Table 3). Most of the previous survival studies focused on *E. coli* O157:H7. Those work showed that the persistence of *E. coli* O157:H7 was largely affected by soil properties (e.g. availability of essential nutrients including carbon, nitrogen, trace elements, salinity, pH and temperature) (Ma et al., 2012a). The persistence of *E. coli* O157:H7 was also influenced by soil biology including indigenous microbial communities (Ma et al., 2013). Potential mechanisms had been shown to include predation, substrate competition and antagonism (Jiang et al., 2002; Ma et al., 2013; Semenov et al., 2007; Unc et al., 2006). It is believed that a soil ecosystem with a lower level of microbial diversity is more sensitive to the invasion of *E. coli* O157:H7 than that with a higher level of biodiversity (Ibekwe et al., 2007; Ibekwe and Ma, 2011; Semenov et al., 2008; van Elsas et al., 2011). Definitely, additional work is needed to correlate the persistence of STEC, especially non-O157 STEC, with the abundance and structure of the indigenous microbial communities. The overall survival of STEC in soils might be a function of a combination of soils' physical, chemical, and biological factors.

In summary, the current study offers additional information on the survival of O157 and non-O157 strains in soils for growing leafy greens, and highlights the longer survival of some non-pathogenic strains in agricultural soils. This study shows that non-O157 STEC can persist in leafy green producing agricultural environments for as long as three months, which might considerably increase the opportunity for their recycling among agricultural soils, cattle, and subsequent transmission into the human food chain. In light of recent epidemiological data, non-O157 STEC should be seen as an emerging risk to be controlled at on-farm preharvest levels. Our findings in the current study suggest therefore, that *E. coli* O157:H7 and non-O157 persisted differently in soils, and point out to the needs for avoidance of over simplifications of survival patterns based on a particular serotype. Therefore, additional caution is required when evaluating the risk associated with STEC as some non-O157 strains carrying virulence genes may persist longer in the farming environment than the common *E. coli* O157 strain.

Conflict of interest

There is no conflict of interest with me and my coauthors.

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