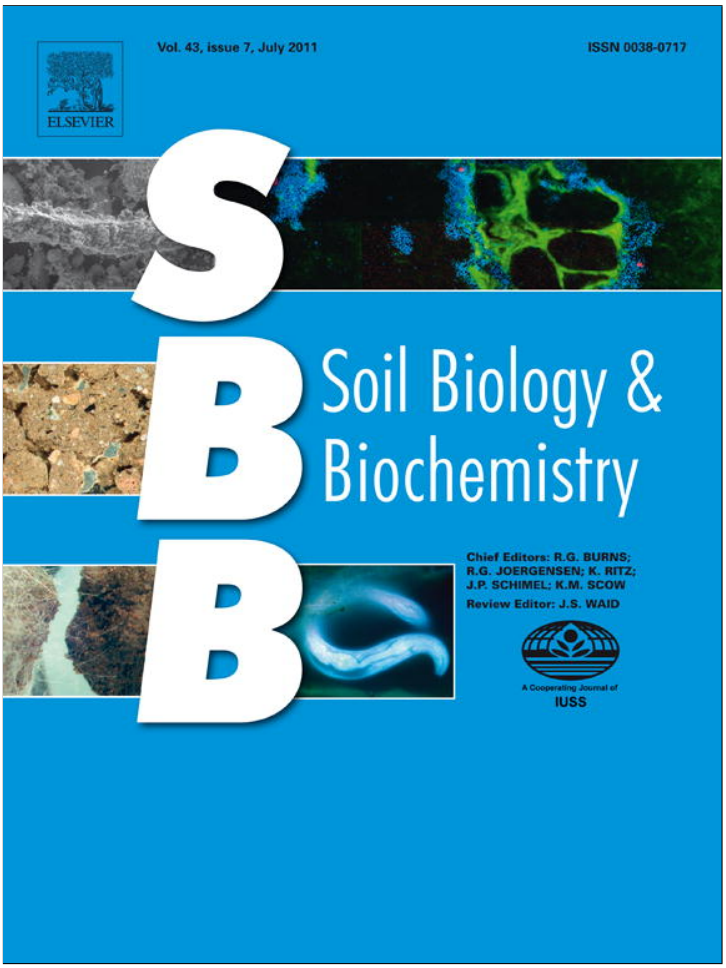

Soil Biology & Biochemistry 43 (2011) 1591–1599

Contents lists available at ScienceDirect

Soil Biology & Biochemistry

journal homepage: www.elsevier.com/locate/soilbio

Survival of *Escherichia coli* in soil with modified microbial community composition

Zhanbei Liang, Zhenli He*, Charles A. Powell, Peter J. Stoffella

Indian River Research and Education Center, Institute of Food and Agricultural Sciences, University of Florida, Fort Pierce, FL 34945, USA

ARTICLE INFO

Article history:
Received 16 November 2010
Received in revised form
2 March 2011
Accepted 12 April 2011
Available online 28 April 2011

Keywords:
Bile salt No. 3
E. coli
Microbial community structure
Soil
Survival

ABSTRACT

Understanding the survival and persistence of *Escherichia coli* in soil with different microbial composition is essential for the accuracy of water quality assessment and microbial source tracking. This microcosm experiment was conducted to investigate the survival pattern of three *E. coli* strains (originated from soil, dog feces and human feces, separately) in soil with modified microbial community composition. Bile salt No. 3 (BS3) of progressively increased density (0.05%, 0.15%, 0.30% and 0.50%) was added into sandy loam soils and incubated for 90 days. Laboratory cultured *E. coli* were then inoculated into soil and incubated for another 150 days to monitor their survival pattern. Change of bacterial community diversity by BS3 was detected by both cultivation based and cultivation independent (PCR-Denaturing Gradient Gel Electrophoresis) methods. In general, progressively increased BS3 concentration resulted in decreased CFU counts both at 10 days and 90 days incubation. DGGE analysis indicated only a slight change in bacterial community composition at 10 days but a significant change at 90 days. Cluster analysis suggested that BS3 treatment grouped separately from controls. Survival of *E. coli* in soil was significantly influenced by the complexity of the microbial community, as die-off rate of *E. coli* progressively declined with the reduction of microbial community diversity. Differential survival of *E. coli* under different soil microbial stress highlights the importance of incorporating biotic factors in predictive models for water quality management and microbial source tracking study.

1. Introduction

To reduce the risk of infection from sewage-borne pathogens in environmental waters, USEPA and WHO developed water quality standards based on the concentration of fecal indicator bacteria because they usually exist in much larger numbers and thus are easier to detect than enteric pathogens. Indicator bacteria, such as *Escherichia coli*, are used as a signal of fecal contamination based on the assumption that the only significant sources of *E. coli* in the environment are feces of human/warm-blooded animals, and *E. coli* has similar survival patterns to those of the pathogenic strains and does not multiply in the environments once shed from the hosts. Multiple recent studies in both tropical and temperate regions, however, suggested that *E. coli* can replicate and reach high densities under favorable conditions outside of hosts (Desmarais et al., 2002; Ishii et al., 2006; Solo-Gabriele et al., 2000), attenuating its suitability as a fecal contamination indicator. Thus, an improved understanding of the response of *E. coli* to a variety of environmental factors is critical for watershed management and regulation criteria development.

Soil is a major non-point source of *E. coli* to water bodies (Ishii et al., 2006; Solo-Gabriele et al., 2000). *E. coli* of agricultural origins enters soil either through the application of animal manures or direct deposition by grazing livestock(Oliver et al., 2006; Topp et al., 2003). Soil bound *E. coli* is transported into water systems via rain/run-off, hence affecting the level of *E. coli* in the water. Survival/persistence of *E. coli* in soil, a key issue when considering the risk of contamination to surrounding waters, is not well defined due to the lack of information concerning the effects of soil factors. Abiotic factors, such as temperature (Berry et al., 1991; McFeters and Terzieva, 1991), moisture (Berry and Miller, 2005; Byappanahalli and Fujioka, 2004; Byappanahalli et al., 2006; Desmarais et al., 2002; Solo-Gabriele et al., 2000), soil texture (Desmarais et al., 2002), organic matter content (Tate, 1978), and manure medium (Oliver et al., 2006), have been shown to influence the survival of *E. coli* in soil, although how these factors affected *E. coli* was unclear. Some biotic factors, such as predation, have also been reported to affect *E. coli* persistence in soil (Brettar and Hofle, 1992). Understanding the effect of other biotic factors, for instance, soil microbial

* Corresponding author. Tel.: +1 772 468 3922; fax: +1 772 468 5668.
E-mail addresses: Liang.Zhanbei@epamail.epa.gov (Z. Liang), zhe@ufl.edu (Z. He), capowell@ufl.edu (C.A. Powell), pjs@ufl.edu (P.J. Stoffella).

0038-0717/$ – see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.soilbio.2011.04.010

community composition, however, is still very limited (Bogosian et al., 1996; Korhonen and Martikainen, 1991).

It is suggested that the survival or persistence of novel microorganisms after their introduction into soil is controlled by the indigenous soil microbial community structure (Colwell, 1997; McGradySteed et al., 1997; Naeem and Li, 1997; Tilman et al., 1997). An inverse relationship was found between the survival rate of *Pseudomonas aeruginosa* and the complexity of wheat rhizospheric microbial diversity (Matos et al., 2005). In another study in artificially constructed microcosms, the invasibility of *Ralstonia solanacearum* biovar 2 was found to be inversely related to the extent of the soil microbial community diversity (Irikiin et al., 2006). Van Elsas (van Elsas et al., 2007) demonstrated that the survival of *Escherichia coli* O157:H7 was negatively affected by the complexity of the soil microbial community. *E. coli* species are genetically diverse (Hartl and Dykhuizen, 1984), and it has been shown that pathogenic strains of *E. coli* survive differently than non-pathogenic strains (Fenlon et al., 2000; Mubiru et al., 2000; Ogden et al., 2001). It is therefore improper to generalize the environmental behavior of pathogenic strains to other *E. coli* populations.

As a selective inhibitory agent, bile salt #3 (BS3) is widely used in microbiological media at the concentration of 0.15% to support the growth of Gram negative enteric bacteria. BS3 has been used in soil microcosm study to lower number of indigenous soil bacteria and thus promote the growth of natural *E. coli* population (Byappanahalli and Fujioka, 2004). In this study BS3 was used to construct soil microcosms with a gradient of microbial community structure, and those microcosms were then used as model soil ecosystems with different microbial community structure to determine the survival of inoculated *E. coli* strains. It is hypothesized that application of BS3 at different concentrations to soil would lead to gradually modified soil microbial community composition, which would inversely affect the survival of the inoculated *E. coli*.

2. Materials and methods

A summary of the experimental scheme used in this study is shown in Fig. 1. Detail of the experimental setting is described as follows:

2.1. Soil sampling and treatment

The sandy soil was freshly collected from the surface 10 cm layer of grassland at the Indian River Research and Education Center, Fort Pierce, Florida. This grassland has been 10 years since its being transformed from a citrus grove and no organic amendments were applied after the transformation. After all stone and plant residues were removed, the soil was homogenized by using a 2-mm sieve, and stored at 4 °C and used within 10 days to set up the soil microcosms. A portion of the soil was sterilized by daily autoclaving for 2 h at 121 °C for 5 successive days following the procedure of Unc and Goss (2006). To facilitate germination of spores between autoclavings the soil sample was kept under aseptic conditions at room temperature. Evaporation loss of water was compensated by adding corresponding amounts of sterile deionized water to soil. Aliquots of autoclaved soil were plate diluted onto nutrient agar plate and incubated at room temperature for 48 h to check the effectiveness of the autoclaving. No growth was detected after 48 h.

The presence/absence of background *E. coli* populations in the collected soil was verified as follows: 1 g of homogenized soil was suspended in 10 ml of tryptic soy broth, shaken and incubated for 24 h at 37 °C (in triplicate). Two hundred μL of the enrichment culture was spread, in duplicate, onto mFC agar plate and incubated at 35 °C for 2 h, followed by 22 h incubation at 44.5 °C. No blue colonies were observed, indicating the absence of background *E. coli* in the soil samples prior to the inoculation.

2.2. Soil microcosms setup

Fresh soils were placed into a sterilized tub. Bile salt solution (20%) was sprayed into the soil and homogenized to a final concentration of 0.50%, 0.30%, 0.15% or 0.05%. Fresh soil without BS3 and sterile soil were used to provide positive and negative controls. Soil moisture contents were adjusted to 50% of its field water capacity using sterilized deionized water, 100 g of soil was placed into a glass vial covered with parafilm and incubated at 25 °C in dark for a total of 90 days to establish a series of soil ecosystems with gradually changed microbial community structure. A total of 594 microcosms (6 treatments × 3 *E. coli* strains × 11 destructive sampling events after the inoculation of *E. coli* × 3 replicate each) were established. Moisture content of all cores was assessed every 5 days and the amount of water lost was added in the form of sterile distilled water via dripping to maintain a constant moisture status within treatments. Microcosms were sampled at day 10 and day 90 to monitor the change of the microbial communities using both culture based and non-culture based methods. Presence or absence of *E. coli* at day 90 was tested using the method described above. No

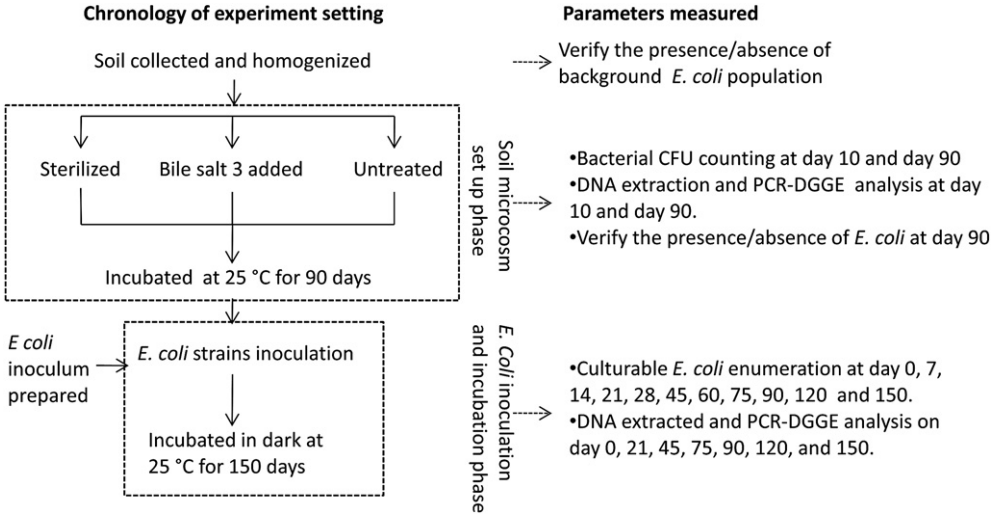

Fig. 1. Experimental scheme used in this study.

growth of *E. coli* was evident at that time point. Soil microcosms at 90 days incubation were used as recipients of *E. coli* inoculation.

2.3. E. coli inoculum preparation and incubation

Strains originally isolated from soil collected from a cattle ranch (S), human feces (H) and dog feces (D) were used as inoculums. Briefly 10 g fresh feces or soil samples were diluted with 95 ml of phosphate buffered saline (PBS; 137 mM NaCl, 2.7 mM KCl, 100 mM Na_2HPO_4, 2 mM KH_2PO_4) and shaken at 280 rpm for 30 min. For feces samples, 200 μL of a 10^{-5} dilution of the upper, solid-matter-free phase were spread onto mFC agar; for soil sample 200 μL of an undiluted soil-free suspension was used. The plates were incubated at 35 °C for 2 h followed by incubation at 44.5 °C for 22 h. Blue colonies were streaked onto mFC agar and incubated at 44.5 °C for 24 h. Single colonies on mFC agar were inoculated onto a MacConkey agar plate and incubated at 37 °C for 24 h. Pink or red colonies on MacConkey agar were confirmed as *E. coli* using a series of biochemical tests (Dombek et al., 2000): growth on minimal lactose agar plates, lack of growth on minimal citrate agar plates and indole production. Confirmed strains were also tested using API20E strips. The three strains used in this study were identified as *E. coli* by API20E with excellent identification scores. Growth curves of the three strains were constructed to compare their growth kinetics using Bioscreen C Automated Microbiology Growth Curve Analysis System (Piscataway, NJ). Very similar growth rates and kinetic values were observed, indicating the comparability of the three strains.

An overnight culture of *E. coli* in nutrient broth was harvested by centrifugation at 3000×*g* and 4 °C for 30 min, the pelleted cells were re-suspended in sterile water and washed twice by centrifugation at 3000×*g* and 4 °C for 30 min, then re-suspended in sterile water to achieve a final concentration of 9 Log CFU/ml, The culture was then maintained at 20 °C for 4 h before inoculation to ensure that any excess nutrients were utilized by the bacteria.

E. coli were inoculated into the soil microcosms via spray and gently mixing to establish a cell density of approximately 8 Log CFU/g dry soil. The use of inoculants at such a high concentration was to ensure the detection of *E. coli* by the subsequent DGGE profiling. Final inoculation density was determined by serial dilution and colony counting. The microcosms were incubated in the dark at 25 °C for a total of 150 days. Soil from microcosms was destructively sampled on day 0 (1 h after inoculation), 7, 14, 21, 28, 45, 60, 75, 90, 120, 150 for the enumeration of culturable *E. coli*. Soil samples were analyzed for the change of microbial community using DGGE on day 0, 21, 45, 75, 90, 120 and 150. Moisture loss during incubation was compensated by adding deionized water to maintain constant soil moisture status.

2.4. Bacterial CFU counts and bacterial indices calculation

Soil samples (5 g) were loaded into 45 ml of sterile phosphate buffered dilution water (PBW) (0.002 M $MgCl_2$, 0.0003 M KH_2PO_4) and sonicated for 30 s on ice using an ultrasonic dismembrator (Model 100, Fisher Scientific). The particle free supernatant was then decimally diluted using sterile PBW. Aliquots of 200 μL of appropriate dilutions were spread in duplicate onto R2A media (Difco) and incubated at room temperature (25 °C) for up to 10 days. Colonies on plates were enumerated daily for the first 6 days and then every other day for the following 4 days. Plates with 30–100 colonies were selected for enumeration. To characterize the bacterial community composition, two indices were calculated. The colony development index (CD) as proposed by Sarathchandra et al. (1997) was calculated as follows: $CD = (N_1/1 + N_2/2 + N_3/3 + N_4/4 + N_5/5 + N_6/6 + N_8/8 + N_{10}/10) \times 100$, where N represents the proportion of bacterial colonies appearing on days 1, 2, 3, …10. It is usually assumed that a high CD value indicates a greater proportion of r-strategist while a low value would suggest a higher proportion of *K*-strategist. As calculated here, CD value ranges from 100 (if all colonies appear on day 1) to 10 (if all colonies appear on day 10). The eco-physiological (EP) index as described by De Leij et al. (1993) was adapted from the concept of Shannon diversity index (H') and usually used to quantify the bacterial colony development on agar (De Leij et al., 1993). EP index was calculated as:$EP = -\sum(P_i \times \log 10 P_i)$, P_i is the population in class I/total population, for each of the eight classes (colonies appearing on each of the 8 days). Higher CD and EP index indicate more even distribution of the colony classes.

2.5. Enumeration of E. coli in incubated soil

Three vials from each treatment were collected for destructive sampling and enumeration of culturable *E. coli* at each time point. Five grams (fresh weight) of soil was added into a 50 ml conical centrifuge tube containing 45 ml of sterile PBW followed by sonication on ice using an ultrasonic dismembrator (Model 100, Fisher Scientific) at set 6 for 30 s. After settling for 10 min the particle free supernatant was decimally diluted and filtered through a membrane filtration system according to standard membrane filtration protocol. Briefly, the solution was filtered through a 0.45 μm membrane filter and incubated on modified membrane-Thermotolerant *E. coli* Agar (Modified mTEC) agar for 2 h at 35 °C followed by incubation at 44.5 °C for 18–22 h. Red or magenta colonies were counted as *E. coli*. As the number of culturable *E. coli* declined below the detection limit of the membrane filtration method (3 log CFU), the MPN method with five tubes per dilution was applied for the enumeration (Clesceri, 1989).

Table 1
Total culturable heterotrophic bacteria (colony forming units, CFU), the eco-physiological index (EP) and colony development index (CD) in soils treated with different level of Bile salt (BS3). CFU were enumerated from R2A media incubated at 25 °C for 48 h. CD was calculated following Sarathchandra et al. (1997) and EP indices as described by De Leij et al. (1993).

Treatments[a]	10 days incubation[b]			90 days incubation		
	CFU count	EP	CD	CFU count	EP	CD
No addition	6.95 ± 0.02a	0.72 ± 0.02a	25.1 ± 3.5a	7.11 ± 0.11a	0.66 ± 0.03a	22.3 ± 2.7a
0.05% BS	5.91 ± 0.31b	0.70 ± 0.01a	26.4 ± 2.8a	6.63 ± 0.05a	0.60 ± 0.01a	24.5 ± 2.5a
0.15% BS	5.07 ± 0.22b	0.64 ± 0.01a	30.7 ± 2.9b	5.93 ± 0.06b	0.58 ± 0.02a	22.1 ± 1.5a
0.30% BS	4.38 ± 0.52bc	0.62 ± 0.01a	33.8 ± 4.2b	5.26 ± 0.13b	0.55 ± 0.02b	25.3 ± 3.1a
0.50% BS	4.02 ± 0.33c	0.51 ± 0.02b	37.3 ± 4.1c	3.92 ± 0.24c	0.42 ± 0.01c	25.1 ± 2.2a
Sterilized (S)	0	N/A[c]	N/A	0	N/A	N/A

[a] BS = Bile salt.
[b] Mean ± SE followed by different letters within a column indicates a significant difference at $P < 0.05$.
[c] N/A = not applicable.

Table 2
Community diversity indices (the Shannon index of diversity (H'), evenness (E) and richness (S)) in soil treated with different density of Bile salt after 10 days and 90 days incubation. The diversity indices were calculated from the relative abundance data obtained from the DGGE banding patterns of the bacterial community.

Treatments	10 days incubation[a]			90 days incubation		
	H'	E	S	H'	E	S
Fresh soil	2.94 ± 0.11a	0.95 ± 0.02a	22 ± 2.1a	3.05 ± 0.05a	0.97 ± 0.02a	23 ± 1.0a
0.05% BS3	2.81 ± 0.06a	0.92 ± 0.01b	21 ± 2.5a	2.55 ± 0.12b	0.86 ± 0.01b	19 ± 2.1b
0.15% BS3	2.80 ± 0.08a	0.95 ± 0.01a	19 ± 1.9a	2.48 ± 0.10b	0.87 ± 0.02b	17 ± 1.3b
0.30% BS3	2.83 ± 0.05a	0.96 ± 0.01a	19 ± 1.0a	2.30 ± 0.09b	0.82 ± 0.01b	16 ± 1.6b
0.50% BS3	2.31 ± 0.10b	0.92 ± 0.02b	12 ± 1.4b	1.94 ± 0.11c	0.84 ± 0.01b	10 ± 1.3c

[a] Mean ± SE followed by different letters within a column indicates a significant difference at $P < 0.05$.

2.6. PCR-DGGE analysis

DNA was extracted from soil using the MobioPowerSoil DNA isolation kit (Mobio Inc., Carlsbad, CA). Modifications were made to increase extraction efficiency and yield (Liang et al., 2008). The yield and fragmentation of the DNA was checked by agarose gel electrophoresis (0.8%), followed by GelStar staining and visualization under UV light. The DNA extract was stored at −20 °C for future use.

Polymerase Chain Reaction-Denaturing gradient gel electrophoresis (PCR-DGGE) analyses were conducted to study the soil microbial community structure. A 566 bp fragment of the V3 region of 16S rRNA was amplified from soil DNA extraction using the eubacterial primer pair 341FGC/907R (BuchholzCleven et al., 1997) in an Eppendorf Mastercycler (Eppendorf). One μL of DNA extract was used as template in a reaction volume of 25 μL containing 1 U Taq DNA polymerase and 1 × PCR buffer (20 mM Tris–HCl, 50 mM KCl, pH 8.4) with 0.3 μg μl^{-1} BSA. The PCR protocol consisted of denaturation at 95 °C for 3 min followed by 27 cycles of 94 °C for 30 s, amplification at 64 °C for 30 s and elongation at 72 °C for 30 s and a final elongation step at 72 °C for 30 min. Amplification products were checked for size and yield by standard 1% (w/v) agarose-0.5× Tris–borate–EDTA (TBE) gel electrophesis with GelStar staining.

Twenty microliters of PCR products were subjected to DGGE in a SE 600 Ruby Standard Dual Cooled Vertical Unit (GE healthcare). The 0.75 mm thick gel containing 8% (w/v) polyacrylamide (37.5:1 acrylamide/bis-acrylamide) and a 40–60% denaturant gradient (100% denaturant is defined as 7 M urea and 40% (v/v) formamide) were electrophoresed for 16 h 30 min at 80 V and 60 °C in 1 × TAE buffer. Gels were stained with GelStar nucleic acid stain and gel images were captured using the Gel Doc XR imaging system (Biorad, Hercules, CA).

Bands of interest were excised from the DGGE gel and stored overnight in 100 μL of water at 4 °C to allow the DNA to passively diffuse out of the gel strips before re-amplification. Ten milliliter of eluted DNA was then used as template with primer pair 341FGC/907R as described above, and the products were subjected to DGGE to check their migration. Confirmed DNA fragments were re-amplified using primer pair 341F/907R and purified from a 1% (w/v) agarose gel using Qiaquick Gel extraction Kit (Qiagene) and cloned into pCR2.1-TOPO vector (Invitrogen). The ligation products were chemically transformed into *E. coli* (strain TOP10) using the TOPO TA Cloning Kit (Invitrogen, http://www.invitrogen.com) following manufacturer instructions. Inserts were sequenced and sequences were compared to the NCBI database using BLASTN (www.ncbi.nlm.nih.gov). DNA from pure culture of the three strains was isolated using UltraClean Microbial DNA Isolation Kit (Mobio Inc., Carlsbad, CA). A 566 bp fragment of the V3 region of 16S rRNA was amplified and sequenced using methods described above.

2.7. Data analysis

E. coli were normalized by transforming to log CFU g^{-1}. The survival rate of *E. coli* in soil (k) was calculated according to the equation: $M_t = M_0 e^{-kt}$, where M_t is the number of bacteria at time t, M_0 is the initial number of bacteria, k is the first order rate coefficient for the net survival rate for bacteria per day, and t is the time in days.

DGGE profiles of all samples were analyzed using GelCompar II (Applied Math) after being normalized by the program and adjusted manually. Each band position present in the gel was binary coded for its presence or absence within a lane and each lane

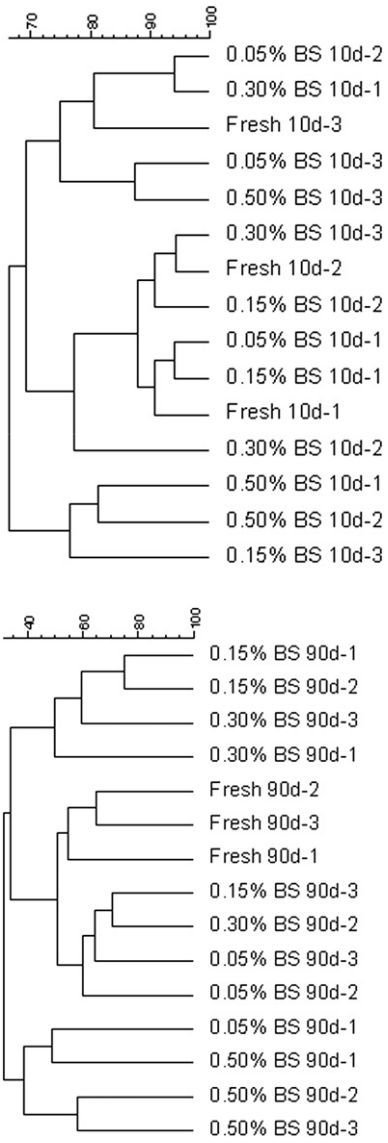

Fig. 2. Clustering of bacterial DGGE patterns in soil with modified microbial communities 10 days (above) and 90 days (below) after incubation with different level of BS3.

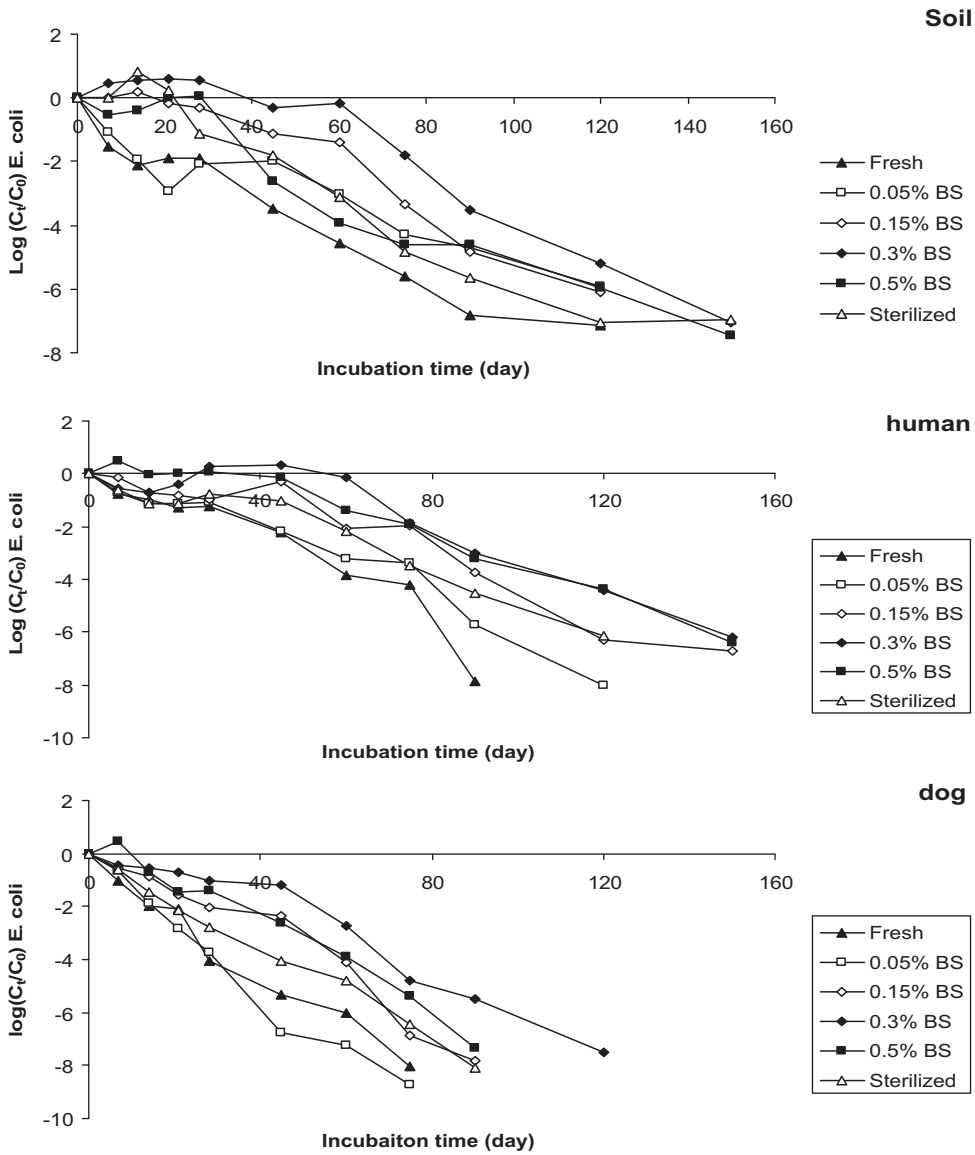

Fig. 3. Survival pattern of three *E. coli* strains in soil treated with BS3 of different levels, Fresh soil and Sterile soil were used as positive and negative control.

was compared by using a similarity matrix displayed as a dendrogram. The clustering algorithm used to calculate the dendrograms was an unweighted pair group method with mathematical averages (UPGMA, Dice coefficient of similarity). Gel images were also converted to densitometric profiles and species (number of band) and their inferred abundance (band intensity) were determined. Species richness (S) refers to the number of bands detected in a given soil sample. Species evenness (E), as a measure of how evenly DGGE bands were distributed in a given soil sample, was calculated as $E = H'/\ln(S)$. To compare changes in diversity of microbial communities with all treatments, the Shannon–Weaver index (H') was calculated from the equation: $H' = -\sum p_i \log p_i$, where $p_i = n_i/N$, n_i is the band intensity of the ith DGGE band and N is the sum of all band intensities in each lane.

3. Results

3.1. Effect of BS3 on microbial community composition

In this study, after treatment with different concentrations of BS3, plate counts were performed to quantify changes in culturable bacteria in soils after 10 days and 90 days incubation. The changes in eco-physiological structure of the bacterial community were indicated by EP and CD indices. The effects of BS3 on the bacterial community diversity were monitored by amplifying fragments of the highly conserved 16S rRNA genes using PCR and profiled by DGGE.

As shown in Table 1, after 10 days incubation, culturable bacteria counts in fresh soils were approximately 7 logs CFU per gram soil. As expected no culturable bacteria were detected in the sterilized soil. CFU counts of the culturable heterotrophic bacteria from all soils with BS3 addition were significantly reduced as compared to soil with no BS3 addition. Increase in the intensity of BS3 treatment led to progressively reduced CFU counts, from about 6 logs CFU per gram soil in the 0.05% BS3 treatment to 4 logs CFU per gram soil in the 0.50% BS3 treatment. Bacteria counts in the 0.05% BS3 treatments were significantly different from the 0.50% BS3 treatments. Culturable bacteria counts in the 0.50% BS3 treatments declined by about 3 logs CFU per gram of soil compared with those in fresh soils. The EP index decreased in soil with BS3 but there was no statistically significant change except for the 0.50% BS3 treatments. In contrast the CD index was significantly increased in soils with BS3 with the exception of the 0.05% BS3 treatments.

Table 3
Decay rate of the three *E. coli* strains in soil with different treatments.

Soil treatments[a]	Human	Soil	Dog	Mean
Fresh	0.056	0.059	0.101	0.072
0.05% BS3	0.064	0.049	0.120	0.078
0.15% BS3	0.045	0.051	0.091	0.062
0.3% BS3	0.041	0.047	0.061	0.050
0.5% BS3	0.043	0.050	0.071	0.055
Sterilized	0.051	0.046	0.086	0.061

[a] BS = Bile salt.

After 90 days incubation, CFU counts in the 0.05%, 0.15% and 0.30% BS3 treatments substantially increased (Table 1), possibly because of the growth of bacteria that adapted to the presence of BS3. However with the exception of the 0.05% BS3 treatment, CFU counts in BS3 treated soils were still significantly lower than those in fresh soil, with up to a 3 logs CFU reduction in the 0.50% BS3 treatments. No significant changes in CFU counts of culturable bacteria were observed in the 0.50% BS3 treatment at day 90 as compared with day 10, indicating that this BS3 level was too high for the recovery of most of the Gram negative bacteria. CFU counts in the 0.15% and 0.30% BS3 treatments were not significantly different from one another. Bacterial community EP indexes decreased in the BS3 treated soil. Unlike those observed after 10 days incubation, EP values in soil with 0.30% and 0.50% BS3 were significantly lower than fresh soil, whereas CD values did not show significant differences among all treatments at day 90.

Contrary to physiological structure, Diversity indexes based on the bacterial community DGGE profiles reflected only a slight change of bacterial diversity at day 10 (Table 2). With the exception of 0.50% BS3 treatment, no significant changes in Shannon diversity index (H') and richness (S) were observed in soils with BS3 addition, compared to those in fresh soil. Evenness (E) in soils with 0.3% and 0.5% BS3 was significantly reduced compared with others. Cluster analysis of the bacterial communities DGGE profile revealed that all treatments were similar, assembled together at about 70% similarity level (Fig. 2a). Noticeably two out of the three replicates of the 0.50% BS3 treatment clearly grouped together with about 80% similarity. No other apparent separation patterns were observed. In contrast after 90 days incubation soil bacterial community structures were significantly changed by BS3 (Table 2). Diversity index H, E and S in soils treated with BS3 were significantly lowered compared with fresh soil, with all three indexes declining with the increase of BS3 intensity. Noticeably, after 90 days incubation, the 0.50% BS3 treatment lost 57% of its DGGE detectable species as compared with that in fresh soil. Cluster analysis of the DGGE profile revealed that bacterial community structures in treated soils were quite different from those in the fresh soil. A very clear dose-dependent effect was observed with two apparent patterns (Fig. 2b): Fresh soils clustered together at a similarity level of about 60%. The 0.50% BS3 treatments clustered separately with 45% similarity, whereas soils with 0.05%, 0.15%, and 0.30% BS3 clustered separately (each with one replicate as an exception) at various similarity levels.

3.2. Dynamics of E. coli strains in soil

Although no obvious growth of *E. coli* was detected, all three *E. coli* strains could persist in soil over an extended period of time, some even remained detectable at the end of the incubation (150d) (Fig. 3). Variation of *E. coli* over time was adapted to examine the dynamic of the inoculated strains in soil microcosms. This approach was often used in most monitoring practices on agricultural fields. Generally, the patterns of survival for all three *E. coli* strains fit quite well with a first order kinetics model, the most commonly used model to describe enteric bacteria die-off in soil (Bolton et al., 1999; Crane and Moore, 1986; Natvig et al., 2002; Reddy et al., 1981), with R square values ranging from 0.821 to 0.937. *E. coli* numbers declined gradually with time with decay rates ranging from 0.04 to 0.12 log CFU per gram of soil per day (Fig. 3). Die-off patterns of *E. coli* in soil are strain dependent: the dog *E. coli* strain decayed more rapidly in soil than the other two strains: over the first 75 days incubation, the level of dog strain declined by up to 8 orders of magnitude, while the decrease of the other two was only two to six orders of magnitude; the dog strain could not be detected from all the treatments after 120 days incubation, while the other two strains demonstrated greater persistence and could be detected in some microcosms even after 150 days incubation.

The survival of *E. coli* in soil was inversely related to the complexity of soil microbial community. In fresh soil without BS3, *E. coli* counts declined progressively and fell below detection levels after 75–120 days incubation (Fig. 3). The decay rate of *E. coli* in fresh soil ranged from 0.056 to 0.102 day^{-1} with a mean k value of 0.072 day^{-1} (Table 3) These decay rates are in the range of values reported by other researchers (Anderson et al., 2005; Habteselassie et al., 2008; Lau and Ingham, 2001) which showed that decay rate was usually in the range of 0.02–0.238 in soil of various types. Although in the 0.05% BS3 treatment *E. coli* died off faster than in fresh soil, decay of *E. coli* was substantially slowed in 0.15%, 0.30% and 0.50% BS3 treatments. With the exception of 0.50% BS3, mean die-off rate declined progressively with the increase of BS3 density. The lowest mean k value, observed in 0.30% BS3 treatments, was only 68% of that in fresh soil. In the 0.50% BS3 treatment, the average die-off rate was 10% higher than that in the 0.30% BS3 treatment.

Survival patterns of *E. coli* in soil were clearly visualized by DGGE band profile of the soil bacterial community. For example, on DGGE profile of soils treated with 0.30% BS3 and inoculated with soil *E. coli* (Fig. 4), one clear band (marked as band A), which was absent in soil with no inoculants, was present in the inoculated soils collected from time 0. The band was sequenced and confirmed to be 100% identical to fragment of the 16S rRNA sequence of this

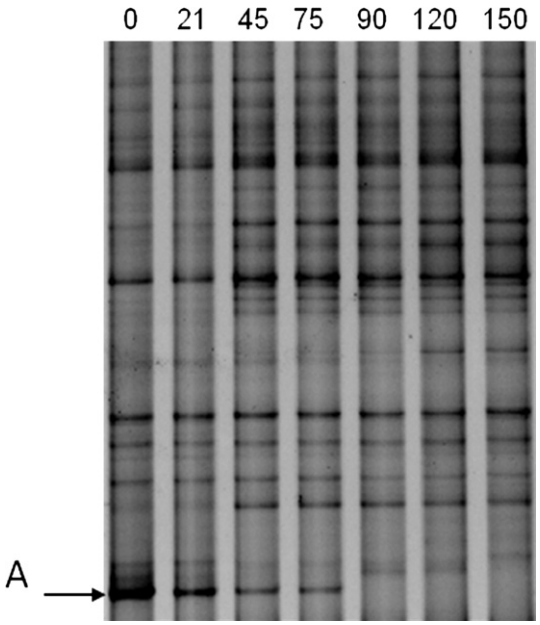

Fig. 4. DGGE fingerprinting of soil treated with 0.3% BS3 and inoculated with *E. coli* of soil origin. The number on top of gel profile indicates time of incubation (days).

soil *E. coli* strain. Thus presence of this band on the DGGE profile indicated the presence of this *E. coli* strain. Intensity of this band decreased with incubation time and became invisible at 90 days and thereafter. DGGE profile for other treatments shared the similar trends but differed on the time when the band indicative of *E. coli* was undetectable. This band was undetectable after 45 days incubation in fresh soil and soil with 0.05% BS3 inoculated with dog *E. coli*. For other soil treatments + *E. coli* combination, usually after 75 or 90 days incubation the band became invisible. This band could not be detected for all samples after 120 days incubation (data not shown).

4. Discussion

This study was designed to assess the survival characteristics of *E. coli* strains in sandy soils with gradually modified microbiota in a microcosm setting when other biotic and abiotic factors remained similar between treatments. Practically this eliminated the impact of other factors on the persistence of *E. coli* when introduced into a new habitat. *E. coli* strains were isolated from three sources and laboratory grown in order to provide relatively comparable inoculation density. Results obtained in this study showed that all three *E. coli* strains tended to survive better in soils with reduced bacterial community diversity.

4.1. Effect of BS3 on microbial community composition

Byappanahalli and Fujioka (2004) found that addition of BS3 to soil microcosms led to 94% to 99% loss of the soil culturable bacteria after 11 days incubation. In our study it was demonstrated that BS3 addition not only significantly reduced bacterial numbers, but also strongly modified the composition of the soil microbial community, as clearly shown by both culture dependent and culture independent methods. The increased CD values in BS3 treated soil at day 10 may suggest that BS3 induced a portion of the bacteria to enter into physiologically inactive status, such as spores or VBNC (viable but non-cultivatable), which in turn relieved the competition for nutrients and thus increased the abundance of bacteria that were adaptive to the presence of BS3. Possible mechanisms involved in the adaption to BS3 may include the induced over-expression of efflux pump genes (Thanassi et al., 1997) that are important for the process of detoxification of intracellular metabolites (Martinez et al., 2009). It is estimated that about 5–10% of all bacterial genes are involved in transport and a large portion of these encode efflux pump (Lomovskaya et al., 2001; Saier and Paulsen, 2001). Non-specific efflux pump may transport a range of structurally dissimilar compounds from within cells into the external environment. Those efflux pump genes may be acquired by either adaptive mutation (Perfeito et al., 2007) or horizontal gene transfer (Droge et al., 1999).

Unlike those obtained by culture based method, only slight changes in the bacterial community composition from BS3 addition was detected at day 10 by DGGE analysis of the amplified 16S rRNA fragments. It is possible that DNA in those VBNC bacteria could still be extracted and fingerprinted using DGGE. The persistence of extractable DNA attached on soil particles after cell decay may also contribute to the similarity of DGGE profile between different treatments at day 10. Only at day 90, a more apparent clustering by treatment was revealed by DGGE. The discrepancies between data from culture and molecular methods may be attributed to the fact that these two methods identify two different spectra of microorganism. Culture methods only detect microorganisms that grow and form colonies on the culture medium under certain incubation conditions. The majority of soil bacteria are unable to grow in culture: an estimate of only 1% of the total bacteria can be cultured routinely in laboratory media (Hirsch et al., 2010). Molecular techniques rely on the efficient extraction of nucleic acid from both culturable and non-cultureable microorganisms in the complex soil matrix and are supposed to detect a much wider spectrum of soil microorganisms. However, bias was often introduced by the DNA based methods because of the presence of inhibitory substances, different amplification efficiency and/or the co-amplification of extracellular DNA fragments.

The concentrations of BS3 used in the present study (0.05–0.5%) were comparable to those used in culture medium (0.15%) and lower than the upper level of BS3 in the human intestinal tract (Rosenberg et al., 2003). Although BS3 might be degraded by some strictly anaerobic enteric bacteria *in vivo* as well as *in vitro* (Aries et al., 1969; Hill and Drasar, 1968), the incubation conditions in our study were not suitable for the fast decay of BS3. Moreover, the decomposition of BS3 was subject to substrate inhibition, and very little reaction was detected when BS3 levels reached 0.50% even if other conditions were kept optimized (Hill and Drasar, 1968). So the inhibition of BS3 on bacterial growth and the modification of the bacterial community might persist in soil microcosms for a long time in our study. This is different from the effect of fumigation on soil biological properties, which usually acts on a one-time basis, leading to the temporarily enhanced availability of nutrients from lysed cells and the fast growth of chloroform survivors.

4.2. Dynamics of E. coli strains in soil

In this study no obvious evidences for growth of *E. coli* were observed, which is not in agreement with studies that suggested that *E. coli* was capable of replicating in the environment (Byappanahalli and Fujioka 1998; Topp et al., 2003; Unc and Goss, 2006). We, however, revealed that all three *E. coli* strains were able to persist in soil for a relatively long time, indicating that soil of this type satisfied the basic nutrient requirements to sustain *E. coli* cell activity. The prolonged population trend in soil has been noted in previous studies (Fenlon et al., 2000; Habteselassie et al., 2008; Jiang et al., 2002; Johannessen et al., 2005; Lau and Ingham, 2001). The differential survivorship between *E. coli* strains, which has been previously described (Anderson et al., 2005; Topp et al., 2003), might reflect the difference in their capability to adapt to the new environment through reduction in cell size, development of starvation resistant forms and/or modification of cell membrane chemistry. Mechanisms including chemical signaling, gene function control and exchange of genetic materials are suggested to be involved in this adaptation (Ben-Jacob, 2003). It might also reveal the different efficiency to utilize resources in the new environments (Koch 1996; Sjogren, 1994; Topp et al., 2003). It is possible that small number of *E. coli* enter into the VBNC status upon the depletion of nutrient and became undetectable by conventional culture methods (Unc et al., 2006). So the un-detection of *E. coli* by plate counting or the MPN method may not necessarily indicate the complete perishing of *E. coli* in the soil microcosms.

Microcosms with different BS3 density treatments differed from each other mainly in two aspects: the BS3 density and the modified microbial community structure in soil. Those differences may, to a large extent, justify the discrepancy in the survival pattern of *E. coli* in microcosms. Interference of indigenous microorganisms for *E. coli* survival has been noted (Korhonen and Martikainen 1991; Unc and Goss, 2006). Diversity of soil microbial communities progressively decreased due to an increase in BS3 density. This might lead to decreased competitive pressure for space and/or nutrients by improving availability of niches and/or resources and decreasing antagonism, which were supposedly conducive for the survival of inoculated *E. coli* (Byappanahalli and Fujioka, 2004). The reduced competitive pressure was suspected to account for the

progressively improved survival of *E. coli* in microcosms with gradually increased BS3 level. This is in agreement with previous reports that ecosystems with lower microbial community complexity were more susceptible to bacterial invasion and more amenable to invaders than those with higher microbial community complexity (Byappanahalli and Fujioka, 1998; Irikiin et al., 2006; Matos et al., 2005; van Elsas et al., 2007). This susceptibility is mainly due to the complicated interactive forces among species (Girvan et al., 2005), which involves microbial signaling, sensing and antibiosis systems. Moreover, the increased density of BS3 may also result in the reduced predation activity of protozoa and thus boost the survival of *E. coli*. However, interactions among microorganisms may also promote mutual growth and persistence (Habteselassie et al., 2008), possibly by enhancing nutrient absorption (Otto et al., 2002) through the production of extracellular enzymes (Maclaren et al., 1984) or other diffusible molecules (Brandl 2006; Loper and Henkels, 1999). Soil microbial community with reduced diversity due to the increased level of BS3 may diminish this beneficial interaction with *E. coli*, thus speeding up its perishing. This was partially supported by the fact that *E. coli* in sterile soil (with the absence of local microbial community) did not survive better than some of the BS3 treatments. That may also be the reason why *E. coli* decayed faster in 0.50% BS3 than in 0.30% BS3. More in-depth research is needed to obtain a more definite answer.

DGGE profile analysis of the soil microbial community clearly reflected the survival patterns of *E. coli* in soil. A band indicative of *E coli* strains was noticeably observed after the inoculation and persisted for an extended period of time. There was, however, discrepancy between the persistence of *E. coli* strain observed by the culture based method and DGGE method. Take the persistence of soil *E. coli* strain in the 0.30% BS3 treatment as an example; DGGE profiling could not detect the *E. coli* affiliated band after 90 days incubation, but culture based methods could still reveal the presence of *E. coli* strain even after 150 days incubation. This inconsistency was due to the detection limit of PCR-DGGE methods: only populations that make up of at least 0.5% of a community can be detected by DGGE (Gelsomino et al., 1999). When the number of *E. coli* dropped below this threshold ratio, those *E. coli* originated 16S rRNA fragments would be outcompeted by fragments from the more abundant populations in the PCR amplification, resulting in the non-detection of *E. coli* in DGGE profile.

4.3. Implication of findings from this study

Determining the persistence pattern of *E. coli* in soil is very important not only for those pathogenic strains which in itself directly represent a health risk when contaminating fresh produce (Solomon et al., 2002), die-off of general strains used as indicators is also a key issue to consider in the area of public health. Those strains may be eventually transferred to water systems by rain/runoff and thus jeopardize the relationship between indicator concentration and human health risk. The differential survival of *E. coli* in soil with different microbial community composition would challenge MST (microbial source tracking) methods that utilize *E. coli* to determine the contribution of various sources to fecal pollution in water. Moreover, survival of some enteric pathogens in soil was parallel with that of *E. coli* (Chandler and Craven 1978, 1980), so clarifying *E. coli* survival behavior may also be useful in predicting the potential risk of enteric pathogen co-entering into soil.

By using BS3 to manipulate the soil bacterial composition we constructed a model microcosm with gradually modified microbial community structure. Our finding that the survival of *E. coli* strains varied in response to different level of microbial stresses in the soil environment is significant in that various agricultural management practices, such as application of fertilizers and herbicides, may affect soil microbial diversity (Ferreira et al., 2009; Jangid et al., 2008) and thus the fate of *E. coli*. Follow up investigations with those more relevant scenarios are under way to confirm the finding from this study. The discrepancy in the survival of *E. coli* emphasizes the difficulties in estimating its behavior in different soil. It should be noted as well that the laboratory study under controlled conditions does not embody the complexity of interaction between environmental processes operating in fields, so laboratory-derived data may fail to represent the field-relevant behavior. For example, when scaling up to field level, the first order survival curve of *E. coli* generated in the laboratory tended to overestimate or underestimate its survival in the real-world scenarios (Oliver et al., 2006), the consequences of depositing manure borne *E. coli* of different genetic types into environmental soil varies because of a complex array of physical, chemical and biological factors that are difficult to simulate (McFeters and Terzieva, 1991). The behavior of fecal indicator under this condition will be very different from what happened in a laboratory microcosm experiment (Park et al., 1991). Further exploration is needed on the persistence of fecal bacteria populations and pathogens under field conditions for the better understanding of their survival potential in the natural soil environments. Scientific knowledge from this study provides valuable information for predictive modeling to improve management plans and mitigate the impact on water quality and the risk to human health.

Acknowledgments

This study was, in part, supported by a grant (contract #4600001774) from South Florida Water Management District, Florida. The author thanks Dr Harwood in University of South Florida for technical assistance. We thank the two anonymous reviewers whose comments helped to improve the manuscript.

References

Anderson, M.L., Whitlock, J.E., Harwood, V.J., 2005. Persistence and differential survival of fecal indicator bacteria in subtropical waters and sediments. Applied and Environmental Microbiology 71, 3041–3048.

Aries, V., Crowther, J.S., Drasar, B.S., Hill, M.J., 1969. Degradation of bile salts by human intestinal bacteria. Gut 10, 575.

Ben-Jacob, E., 2003. Bacterial self-organization: co-enhancement of complexification and adaptability in a dynamic environment. Philosophical Transactions of the Royal Society of London Series A-Mathematical Physical and Engineering Sciences 361, 1283–1312.

Berry, E.D., Miller, D.N., 2005. Cattle feedlot soil moisture and manure content: II. Impact on *Escherichia coli* O157. Journal of Environmental Quality 34, 656–663.

Berry, C., Lloyd, B.J., Colbourne, J.S., 1991. Effect of heat-shock on recovery of *Escherichia coli* from drinking-water. Water Science and Technology 24, 85–88.

Bogosian, G., Sammons, L.E., Morris, P.J.L., Oneil, J.P., Heitkamp, M.A., Weber, D.B., 1996. Death of the *Escherichia coli* K-12 strain W3110 in soil and water. Applied and Environmental Microbiology 62, 4114–4120.

Bolton, D.J., Byrne, C.M., Sheridan, J.J., McDowell, D.A., Blair, I.S., 1999. The survival characteristics of a non-toxigenic strain of *Escherichia coli* O157: H7. Journal of Applied Microbiology 86, 407–411.

Brandl, M.T., 2006. Fitness of human enteric pathogens on plants and implications for food safety. Annual Review of Phytopathology 44, 367–392.

Brettar, I., Hofle, M.G., 1992. Influence of ecosystematic factors on survival of *Escherichia coli* after large-scale release into lake water mesocosms. Applied and Environmental Microbiology 58, 2201–2210.

BuchholzCleven, B.E.E., Rattunde, B., Straub, K.L., 1997. Screening for genetic diversity of isolates of anaerobic Fe(II)-oxidizing bacteria using DGGE and whole-cell hybridization. Systematic and Applied Microbiology 20, 301–309.

Byappanahalli, M.N., Fujioka, R.S., 1998. Evidence that tropical soil environment can support the growth of *Escherichia coli*. Water Science and Technology 38, 171–174.

Byappanahalli, M., Fujioka, R., 2004. Indigenous soil bacteria and low moisture may limit but allow faecal bacteria to multiply and become a minor population in tropical soils. Water Science and Technology 50, 27–32.

Byappanahalli, M.N., Whitman, R.L., Shively, D.A., Sadowsky, M.J., Ishii, S., 2006. Population structure, persistence, and seasonality of autochthonous *Escherichia*

coli in temperate, coastal forest soil from a Great Lakes watershed. Environmental Microbiology 8, 504–513.
Chandler, D.S., Craven, J.A., 1978. Environmental-factors affecting *Escherichia coli* and *Salmonella typhimurium* numbers on land used for effluent disposal. Australian Journal of Agricultural Research 29, 577–585.
Chandler, D.S., Craven, J.A., 1980. Relationship of soil-moisture to survival of *Escherichia coli* and *Salmonella typhimurium* in soils. Australian Journal of Agricultural Research 31, 547–555.
Clesceri, L.S., 1989. Standard Methods for the Examination of Water and Wastewater, seventieth ed. American Public Health Association, Washington, DC., USA.
Colwell, R.R., 1997. Microbial diversity: the importance of exploration and conservation. Journal of Industrial Microbiology & Biotechnology 18, 302–307.
Crane, S.R., Moore, J.A., 1986. Modeling enteric bacterial die-off – a review. Water Air and Soil Pollution 27, 411–439.
De Leij, F.A.A.M., Whipps, J.M., Lynch, J.M., 1993. The use of colony development for the characterization of bacterial communities in soil and on roots. Microbial Ecology 27, 81–97.
Desmarais, T.R., Solo-Gabriele, H.M., Palmer, C.J., 2002. Influence of soil on fecal indicator organisms in a tidally influenced subtropical environment. Applied and Environmental Microbiology 68, 1165–1172.
Dombek, P.E., Johnson, L.K., Zimmerley, S.T., Sadowsky, M.J., 2000. Use of repetitive DNA sequences and the PCR to differentiate *Escherichia coli* isolates from human and animal sources. Applied and Environmental Microbiology 66, 2572–2577.
Droge, M., Puhler, A., Selbitschka, W., 1999. Horizontal gene transfer among bacteria in terrestrial and aquatic habitats as assessed by microcosm and field studies. Biology and Fertility of Soils 29, 221–245.
Fenlon, D.R., Ogden, I.D., Vinten, A., Svoboda, I., 2000. The fate of *Escherichia coli* and *E coli* O157 in cattle slurry after application to land. Journal of Applied Microbiology 88, 149S–156S.
Ferreira, E.P.D., Dusi, A.N., Costa, J.R., Xavier, G.R., Rumjanek, N.G., 2009. Assessing insecticide and fungicide effects on the culturable soil bacterial community by analyses of variance of their DGGE fingerprinting data. European Journal of Soil Biology 45, 466–472.
Gelsomino, A., Keijzer-Wolters, A.C., Cacco, G., van Elsas, J.D., 1999. Assessment of bacterial community structure in soil by polymerase chain reaction and denaturing gradient gel electrophoresis. Journal of Microbiological Methods 38, 1–15.
Girvan, M.S., Campbell, C.D., Killham, K., Prosser, J.I., Glover, L.A., 2005. Bacterial diversity promotes community stability and functional resilience after perturbation. Environmental Microbiology 7, 301–313.
Habteselassie, M., Bischoff, M., Blume, E., Applegate, B., Reuhs, B., Brouder, S., Turco, R.F., 2008. Environmental controls on the fate of *Escherichia coli* in soil. Water Air and Soil Pollution 190, 143–155.
Hartl, D.L., Dykhuizen, D.E., 1984. The population-genetics of *Escherichia coli*. Annual Review of Genetics 18, 31–68.
Hill, M.J., Drasar, B.S., 1968. Degradation of bile salts by human intestinal bacteria. Gut 9, 22.
Hirsch, P.R., Mauchline, T.H., Clark, I., 2010. Culture-independent molecular techniques for soil microbial ecology. Soil Biology & Biochemistry 42, 878–887.
Irikiin, Y., Nishiyama, M., Otsuka, S., Senoo, K., 2006. Rhizobacterial community-level, sole carbon source utilization pattern affects the delay in the bacterial wilt of tomato grown in rhizobacterial community model system. Applied Soil Ecology 34, 27–32.
Ishii, S., Ksoll, W.B., Hicks, R.E., Sadowsky, M.J., 2006. Presence and growth of naturalized *Escherichia coli* in temperate soils from lake superior watersheds. Applied and Environmental Microbiology 72, 612–621.
Jangid, K., Williams, M.A., Franzluebbers, A.J., Sanderlin, J.S., Reeves, J.H., Jenkins, M.B., Endale, D.M., Coleman, D.C., Whitman, W.B., 2008. Relative impacts of land-use, management intensity and fertilization upon soil microbial community structure in agricultural systems. Soil Biology & Biochemistry 40, 2843–2853.
Jiang, X.P., Morgan, J., Doyle, M.P., 2002. Fate of *Escherichia coli* O157: H7 in manure-amended soil. Applied and Environmental Microbiology 68, 2605–2609.
Johannessen, G.S., Bengtsson, G.B., Heier, B.T., Bredholt, S., Wasteson, Y., Rorvik, L.M., 2005. Potential uptake of *Escherichia coli* O157: H7 from organic manure into crisphead lettuce. Applied and Environmental Microbiology 71, 2221–2225.
Koch, A.L., 1996. What size should a bacterium be? A question of scale. Annual Review of Microbiology 50, 317–348.
Korhonen, L.K., Martikainen, P.J., 1991. Survival of *Escherichia coli* and *Campylobacter jejuni* in untreated and filtered lake water. Journal of Applied Bacteriology 71, 379–382.
Lau, M.M., Ingham, S.C., 2001. Survival of faecal indicator bacteria in bovine manure incorporated into soil. Letters in Applied Microbiology 33, 131–136.
Liang, Z.B., Drijber, R.A., Lee, D.J., Dwiekat, I.M., Harris, S.D., Wedin, D.A., 2008. A DGGE-cloning method to characterize arbuscular mycorrhizal community structure in soil. Soil Biology & Biochemistry 40, 956–966.
Lomovskaya, O., Warren, M.S., Lee, A., 2001. Identification and characterization of inhibitors of multidrug resistance efflux pumps in Pseudomonas aeruginosa: novel agents for combination therapy. Antimicrobial Agents and Chemotherapy 45, 105–116.
Loper, J.E., Henkels, M.D., 1999. Utilization of heterologous siderophores enhances levels of iron available to *Pseudomonas putida* in the rhizosphere. Applied and Environmental Microbiology 65, 5357–5363.
Maclaren, D.M., Namavar, F., Verweijvanvught, A., Vel, W.A.C., Kaan, J.A., 1984. Pathogenic synergy – mixed intra-abdominal infections. Antonie Van Leeuwenhoek Journal of Microbiology 50, 775–787.
Martinez, J.L., Sanchez, M.B., Martinez-Solano, L., Hernandez, A., Garmendia, L., Fajardo, A., Alvarez-Ortega, C., 2009. Functional role of bacterial multidrug efflux pumps in microbial natural ecosystems. Fems Microbiology Reviews 33, 430–449.
Matos, A., Kerkhof, L., Garland, J.L., 2005. Effects of microbial community diversity on the survival of *Pseudomonas aeruginosa* in the wheat rhizosphere. Microbial Ecology 49, 257–264.
McFeters, G.A., Terzieva, S.I., 1991. Survival of *Escherichia coli* and *Yersinia enterocolitica* in stream water – comparison of field and laboratory exposure. Microbial Ecology 22, 65–74.
McGradySteed, J., Harris, P.M., Morin, P.J., 1997. Biodiversity regulates ecosystem predictability. Nature 390, 162–165.
Mubiru, D.N., Coyne, M.S., Grove, J.H., 2000. Mortality of *Escherichia coli*O157: H7 in two soils with different physical and chemical properties. Journal of Environmental Quality 29, 1821–1825.
Naeem, S., Li, S.B., 1997. Biodiversity enhances ecosystem reliability. Nature 390, 507–509.
Natvig, E.E., Ingham, S.C., Ingham, B.H., Cooperband, L.R., Roper, T.R., 2002. *Salmonella enterica* serovar *typhimurium* and *Escherichia coli* contamination of root and leaf vegetables grown in soils with incorporated bovine manure. Applied and Environmental Microbiology 68, 2737–2744.
Ogden, I.D., Fenlon, D.R., Vinten, A.J.A., Lewis, D., 2001. The fate of *Escherichia coli* O157 in soil and its potential to contaminate drinking water. International Journal of Food Microbiology 66, 111–117.
Oliver, D.M., Haygarth, P.M., Clegg, C.D., Heathwaite, L., 2006. Differential *E coli* die-off patterns associated with agricultural matrices. Environmental Science & Technology 40, 5710–5716.
Otto, B.R., van Dooren, S.J.M., Dozois, C.M., Luirink, J., Oudega, B., 2002. *Escherichia coli* hemoglobin protease autotransporter contributes to synergistic abscess formation and heme-dependent growth of *Bacteroides fragilis*. Infection and Immunity 70, 5–10.
Park, R.W.A., Griffiths, P.L., Moreno, G.S., 1991. Sources and survival of campylobacters – relevance to enteritis and the food-industry. Journal of Applied Bacteriology 70, S97–S106.
Perfeito, L., Fernandes, L., Mota, C., Gordo, I., 2007. Adaptive mutations in bacteria: high rate and small effects. Science 317, 813–815.
Reddy, K.R., Khaleel, R., Overcash, M.R., 1981. Behavior and transport of microbial pathogens and indicator organisms in soils treated with organic wastes. Journal of Environmental Quality 10, 255–266.
Rosenberg, E.Y., Bertenthal, D., Nilles, M.L., Bertrand, K.P., Nikaido, H., 2003. Bile salts and fatty acids induce the expression of Escherichia coli AcrAB multidrug efflux pump through their interaction with Rob regulatory protein. Molecular Microbiology 48, 1609–1619.
Saier, M.H., Paulsen, I.T., 2001. Phylogeny of multidrug transporters. Seminars in Cell & Developmental Biology 12, 205–213.
Sarathchandra, S.U., Burch, G., Cox, N.R., 1997. Growth patterns of bacterial communities in the rhizoplane and rhizosphere of white clover (*Trifoliumrepens L.*) and perennial ryegrass (*Loliumperenne L.*) in long-term pasture. Applied Soil Ecology 6, 293–299.
Sjogren, R.E., 1994. Prolonged survival of an environmental *Escherichia coli* in laboratory soil microcosms. Water Air and Soil Pollution 75, 389–403.
Solo-Gabriele, H.M., Wolfert, M.A., Desmarais, T.R., Palmer, C.J., 2000. Sources of *Escherichia coli* in a coastal subtropical environment. Applied and Environmental Microbiology 66, 230–237.
Solomon, E.B., Yaron, S., Matthews, K.R., 2002. Transmission of *Escherichia coli*O157: H7 from contaminated manure and irrigation water to lettuce plant tissue and its subsequent internalization. Applied and Environmental Microbiology 68, 397–400.
Tate, R.L., 1978. Cultural and environmental-factors affecting longevity of *Escherichia coli* in histosols. Applied and Environmental Microbiology 35, 925–929.
Thanassi, D.G., Cheng, L.W., Nikaido, H., 1997. Active efflux of bile salts by *Escherichia coli*. Journal of Bacteriology 179, 2512–2518.
Tilman, D., Knops, J., Wedin, D., Reich, P., Ritchie, M., Siemann, E., 1997. The influence of functional diversity and composition on ecosystem processes. Science 277, 1300–1302.
Topp, E., Welsh, M., Tien, Y.C., Dang, A., Lazarovits, G., Conn, K., Zhu, H., 2003. Strain-dependent variability in growth and survival of *Escherichia coli* in agricultural soil. FEMS Microbiology Ecology 44, 303–308.
Unc, A., Goss, M.J., 2006. Culturable *Escherichia coli* in soil mixed with two types of manure. Soil Science Society of America Journal 70, 763–769.
Unc, A., Gardner, J., Springthorpe, S., 2006. Recovery of *Escherichia coli* from soil after addition of sterile organic wastes. Applied and Environmental Microbiology 72, 2287–2289.
van Elsas, J.D., Hill, P., Chronakova, A., Grekova, M., Topalova, Y., Elhottova, D., Kristufek, V., 2007. Survival of genetically marked *Escherichia coli*O157: H7 in soil as affected by soil microbial community shifts. ISME Journal 1, 204–214.