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REVIEW ARTICLE

Fate of *Escherichia coli* O157:H7 and *Salmonella enterica* in the manure-amended soil-plant ecosystem of fresh vegetable crops: A review

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Abstract

Enterohemorrhagic *Escherichia coli* (EHEC) and *Salmonella enterica* have been implicated in several disease outbreaks linked to consumption of fresh vegetables. Both ruminant and non-ruminant animals carry EHEC and *S. enterica* in their gastrointestinal tracts and can shed the pathogens in the faecal matter both in symptomatic and asymptomatic states. Application of animal waste in soil fertility management and irrigation of crops with contaminated waste water has been recognised as an important route through which EHEC and *S. enterica* can contaminate fresh vegetables during primary production. The behavior of *E. coli* O157:H7 and *S. enterica* in the agricultural environment has been extensively studied in the last decades. Several microbiological detection methods have been applied. This review therefore puts together current knowledge on the behavior of *E. coli* O157:H7 and *S. enterica* in the manure-amended soil-plant ecosystem of fresh vegetable crops during cultivation under various environmental conditions. The review focuses on methodological issues involved in undertaking survival studies and makes comparative analysis of experimental results obtained from studies conducted under controlled environmental conditions integrating results obtained from field experiments. Finally, a theoretical discussion on the potential likely impact of climate change on pre-harvest safety of field-cultivated vegetables is highlighted.

Keywords

Enterohemorrhagic *Escherichia coli*, fate, fresh vegetable crops, manure-amended soil-plant ecosystem, *Salmonella enterica*

History

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Introduction

Microbiological food-borne illnesses linked to consumption of fresh fruits and vegetables continue to be a public health problem (Akhtar et al., 2012; Barak et al., 2005; Doyle & Erickson, 2008) albeit the ever increasing demand for fresh agricultural products internationally (Heaton & Jones, 2008). On the other hand, development and rapid growth in fast-food service enterprises particularly in urban centres as seen throughout the world today, coupled with a change in peoples' eating habits in favor of an increased intake of fresh fruits and vegetables, suggest strongly that demand for fresh produce will continue to increase. One major factor driving the demand for fresh fruits and vegetables is the increase in consumers' awareness of the potential nutritional and health benefits associated with the consumption of fresh produce (Everis, 2004). This shift in consumer behavior is supported by results from epidemiological studies which show that increased consumption of fresh fruits and vegetables is highly associated with a reduced risk of cancer and coronary heart diseases (Dauchet et al., 2006; He et al., 2007; O'Sullivan, 1998; Svendsen et al., 2007). In order to design strategies that can adequately guarantee public safety from microbiological hazards associated with fresh fruits and vegetables, a comprehensive understanding of the behavior of potential pathogens along the production chain of fresh produce is paramount. Such an understanding should take a global perspective because produce safety should be considered a global issue considering that large volumes of fresh produce traded on the international market originate from diverse climatic and geographical regions.

Fresh fruits and vegetables can be contaminated with various human pathogens including bacteria, protozoa and viruses (De Rover, 1998). The norovirus (NoV) and hepatitis A virus (HAV) are the most important food-borne viruses recognized so far (Koopmans & Duiz, 2004) although their association with fresh produce is not yet well established. *Gardia lambia*, *Cyclospora cayetanensis* and *Cryptosporidium parvum* are protozoal parasites of great concern (Ortega et al., 1997; Sterling & Ortega, 1999). Bacterial pathogens of high importance include *Shigella* spp.,

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Salmonella spp., enterotoxigenic and enterohemorrhagic *Escherichia coli*, *Campylobacter* spp., *Listeria monocytogenes*, *Yersinia enterocolitica*, *Bacillus cereus*, *Clostridium botulinum* (Beuchat, 1996; Brackett, 1999), *Enterobacter sakazakii* (Kim & Beuchat, 2005) and *Aeromonas hydrophila* (Daskalov, 2006). Among the bacterial pathogens, the most common etiologic agents of disease outbreaks linked to consumption of fresh vegetables are the *E. coli* O157:H7 and *S. enterica* (Mootian et al., 2009; Olaimat & Holley, 2012). These organisms are particularly important because of their low infectious doses (Chart, 2000; Darwin & Miller, 1999; Tilden et al., 1996) and their ability to survive under refrigeration conditions (De Rover, 1998). *Escherichia coli* O157:H7 and *S. enterica* are known to inhabit the gastrointestinal tracts of both ruminant and non-ruminant livestock (Feder et al., 2003; Grimont et al., 2000; Michele et al., 2007; Naylor et al., 2003; Sánchez-Jiménez & Cardona-Castro, 2004; Vanselow et al., 2005; Willis et al., 2002). Fecal shading of the two organisms by the animals has been reported to occur both in symptomatic and asymptomatic states (Cummings et al., 2009; Faith et al., 1996; Losinger et al., 1995; Omisakin et al., 2003).

Although contamination of fresh vegetables with human pathogens can occur at any point along the farm-to-consumption handling chain, however, the use of untreated livestock waste and/or contaminated irrigation water in primary production has been recognized as a principal direct route of fresh vegetable contamination with *E. coli* O157:H7 and *S. enterica* at pre-harvest stage (Beuchat, 2002). Pre-harvest contamination is particularly important because research has so far shown that all known food-grade post-harvest sanitizers have limited efficacies on target pathogenic organisms present on/in fresh vegetables (Beuchat & Ryu, 1997; Beuchat, 1998, 1999; Shirron et al., 2009; Taormina & Beuchat, 1999). Of great importance are cells of pathogenic organisms that are internalised in plant tissues (Itoh et al., 1998; Jablasone et al., 2005; Solomon et al., 2002; Warriner et al., 2003a) or immobilised in a biofilm matrix on the plant surface (Warner et al., 2008) since such localities have been shown to offer cells protection from sanitizers (Dong et al., 2003; Itoh et al., 1998; Solomon et al., 2002; Takeuchi & Frank, 2000, 2001). Moreover, it has been shown that the use of chemical decontamination techniques can generate potentially toxic by-products (Guten, 2003; Hua & Reckhow, 2007; Jyoti & Pandit, 2004; Lee et al., 2004; Ölmez & Kretzschmar, 2009; Richardson, 2003; Richardson et al., 1994; Shen et al., 2010; Simons & Sanguansri, 1997). Due to potential human health risks associated with disinfection by-products, there have been calls to use potable water instead to clean fresh produce (Ongeng et al., 2006). However, the use of potable water to clean vegetables would inevitably require that fresh produce should be microbiologically sound with respect to pathogens right from the field if such an approach is to be meaningful, since vigorous washing of vegetables with potable water typically reduces the microbial load by only 1–2 logs and is therefore insufficient to guarantee microbial safety (Beuchat, 1998; Beuchat et al., 2001, 2004; Gonzalez et al., 2004; Kim et al., 2006; Wang et al., 2006; Ruiz-Cruz et al., 2007).

In addition, interest in the application of bacteriophage-mediated biocontrol of food-borne pathogens on fresh produce and other ready-to-eat foods postharvest has emerged (Goodridge & Bisha, 2011). However, results of several experiments conducted so far with fresh produce show that the efficacy of the phage technology is just modest and cannot guarantee the safety of fresh produce because all results are based on experiments conducted with test organisms inoculated on the surface of vegetables, while the efficacy of the phage technology on pathogens internalized in plant tissues has not been demonstrated (Carter et al., 2012 and references therein).

Recent reviews in the subject area have focused on general microbiological food safety situation in developing countries (Akhtar et al., 2012), fundamental aspects of *E. coli* survival in the environment (van Elsas et al., 2011), the role of biofilm formation on colonisation and survival of *S. enterica* on plant leaves (Steenackers et al., 2011), interaction of enteric bacteria with phytobacteria (Teplitski et al., 2011) and general ecological aspects of *Salmonella* survival (Jacobsen & Bech, 2011). In this review, we put together knowledge available in literature, on the behavior of *E. coli* O157:H7 and *S. enterica* in manure, manure-amended soil and in the manure-amended-soil-plant ecosystem of fresh vegetable crops under various environmental conditions. We start by looking at methodological constraints hindering experimental studies on the fate of *E. coli* O157:H7 and *S. enterica* in the agricultural environment. We then focus on comparative analysis of experimental results obtained from controlled environmental-setups integrating results obtained from field studies, and highlight the likely impact of climate change on the behavior of manure-borne pathogenic bacteria in the agricultural environment.

Methodological constraints hindering experimental studies on survival

The success of survival studies performed in natural ecosystems such as the manure-amended-soil–plant complex critically depends on accurate monitoring of the test strains introduced in the matrix. However, in practice, key elements such as installation and operational costs, accuracy and environmental and safety considerations dictate the choice of the microbiological methods required. Quantitative polymerase chain reaction (qPCR) has been used to quantify the populations of the introduced test strains in various survival studies performed in vitro (Garcìa et al., 2010; Ibekwe et al., 2002; Ibekwe & Grieve, 2003; Novinsack et al., 2007). The rationale for the application of qPCR is mainly based on the premise that the technique can quantify both the culturable and the viable but non-culturable (VBNC) fractions of the test strains under investigation. However, qPCR methodology has several accuracy-related pitfalls. The negative impact of PCR inhibitors on DNA amplification (Monterio et al., 1997; Tebbe & Vahjen, 1993; van Wintzingerode et al., 1997; Wilson, 1997) and lack of an efficient method for extracting nucleic acids from environmental samples (Artz et al., 2006; Klerks et al., 2006; Martin-Laurent et al., 2001) culminate in underestimation of cell counts. On the other hand, the requirement for an enrichment step (Marsh et al., 1998; Klerks et al., 2006) and the fact that DNA can persist longer after cell death (Artz et al., 2006; Ibekwe et al., 2002;

Josephson et al., 1993) inevitably lead to overestimation of cell counts and/or survival time. Whereas the incorporation of selective nucleic acid intercalating dyes (e.g. ethidium monoazide and propidium monoazide) to the qPCR methodology can improve accuracy by reducing signals from DNA originating from dead cells (Cawthorn & Witthuhn, 2008; Nocker & Camper, 2009; Rudi et al., 2005), the poor detection limit of the qPCR technique for organisms from environmental samples (3 log CFU/g: Ibekwe et al., 2002, 5 log CFU/g: Artz et al., 2006, 4 log CFU/g: Jacobsen & Holben, 2007; Arthurson et al., 2011) still limits the applicability of PCR-based technique in survival studies. In addition, qPCR methodology requires expensive equipment which is usually not available in some laboratories especially in developing countries.

Fluorescent in situ hybridization (FISH) is another technique developed for microbial detection through targeting of rRNA (Lepeuple et al., 2003; Regnault et al., 2000; Rompré et al., 2002). However, failure of the FISH technique to distinguish target (FISH-labelled) cells from autofluorescent particles present in the background substratum (Hahn et al., 1993; Rogers et al., 2007; Schönholzer et al., 2002; Zarda et al., 1997) makes the technique unsuitable in survival studies. Secondly, the low level of rRNA that normally occurs in stressed cells culminates in diminished level of fluorescence, which presents difficulty in the enumeration of the target organism (Wagner et al., 2003). Although the inclusion of the catalysed reporter deposition dye to the traditional FISH methodology substantially improved the detection of the hybridized cells introduced into the soil (Eickhorst & Tippkötter, 2008), the dependence of FISH procedure on epifluorescence microscopy or flow cytometry makes the technique prohibitively expensive for use in survival studies especially in developing countries where laboratories are in most cases ill-equipped. In addition, FISH technique has a poor detection limit, which is undesirable in survival studies.

An alternative approach is the use of reporter genes. Such genes can be stably introduced into the strain of interest and used to monitor the strain directly by its phenotype or indirectly after plating. Often used are the bioluminescence reporter genes, i.e. *luc* and *lux* (Artz et al., 2006; Duncan et al., 1994; Fratamico et al., 1997; Meikle et al., 1994, 1995; Ritchie et al., 2003; Unge et al., 1999; Warriner et al., 2003a). The *lux* gene encodes for the bacterial luciferase whose activity depends on the level of $FMNH_2$ (Unge et al., 1999) while the *luc* gene encodes for eukaryotic luciferase whose activity depends on ATP instead (deWet et al., 1985). One disadvantage of the bioluminescence reporter gene systems in survival studies is their specific substrate requirement for detectable light emission (*n*-decanal for bacterial luciferase and luciferin for eukaryotic luciferase). Secondly, the *luc* or *lux*-marked organisms cannot be used as test strains in field studies in respect of the regulations that prohibit the utilisation of genetically modified organisms (GMOS) in field studies. The high detection limit of bioluminescence technique for organisms from environmental samples also limits its applicability in survival studies in case direct measurements are done. For instance, potential luminescence of the chromosomally *lux* marked-*E. coli* O157:H7 was substantially masked in soil samples and consequently the technique exhibited a detection limit of 4 log CFU/g (Ritchie et al., 2003), which is rather high compared to detection limit of 2 log CFU/g which is normally encountered in surface-spread plate count method.

The green fluorescent protein (GFP) reporter genes have also been used for monitoring cell numbers in conjunction with microscopy (Skillman et al., 1998), flow cytometry or culture method (Fratamico et al., 1997, Tombolini et al., 1997). GFP as a marker is widely preferred due to the non-species dependence characteristic and lack of requirement for any substrates or co-factors (Chalfie et al., 1994). These properties enable microscopic detection of the target organism in a non-destructive manner (Heim et al., 1994). However, the high degree of stability of GFP can lead to overestimation of cell counts during microscopy or flow cytometry since fluorescence can persist even after cell death (Skillman et al., 1998). The fluorescent nucleic acid stain propidium iodide (PI) which only enters cells with damaged cell membrane, is a counter stain which when used in combination with GFP gives more reproducible results by distinguishing between fluorescent dead and fluorescent viable cells (Banning et al., 2002). However, as for the *lux*/*luc* genes, it is worth noting that the PI-GFP technique, though elegant, cannot be used in field studies in respect of environmental regulation in many countries which prohibit the release of GMOS in the field. PI-GFP technique is also expensive due to mandatory requirement for a fluorescent microscope or a flow cytometer which may not be readily available in laboratories in developing countries. The GFP technique in combination with plate count method is a cheaper alternative to the PI-GFP approach. But still, restriction on field release of GMOS makes the use of GFP in plate counting unfit for use in field studies. Thus the bulk of studies on environmental survival of *E. coli* O157:H7 and *S. typhimurium* that used the GFP technique in conjunction with plate counting have therefore been performed in the laboratory (Franz et al., 2005, 2007a,b, 2008; Islam et al., 2004a; Jiang et al., 2002; Natvig et al., 2002; Semenov et al., 2007, 2008, 2009). Results obtained from such laboratory experiments may not be realistic considering the fact that environmental factors that affect survival of organisms fluctuate a lot in the field and cannot be accurately simulated in the laboratory as attempted by Semenov et al. (2007). This is particularly important in situations where vegetable production takes place entirely in the field (especially in developing countries). Other reporter genes such as *lac*Z (encoding for β-galactosidase), *cat* (encoding for chloramphenicol acetyltransferase) and *gus* (encoding for β-glucuronidase) are less suitable for survival studies due to the potential for background of these genes in indigenous populations thus leading to false positive results.

With respect to the regulations that limit the use of GMO in field studies, some field experiments have been carried out using non-genetically-modified strains of *E. coli* O157:H7 and *Salmonella* sp. while applying a culture-dependent method to monitor the population size of the introduced test strains (Avery et al., 2004a,b; Bolton et al., 1999; Hutchison et al., 2004, 2005; You et al., 2006). Although the approach used in those studies was consistent with the requirement regarding the preservation of environmental integrity, however, realization of accurate results was impeded by difficulty in monitoring the population of the introduced test strains in complex mixed populations. This was so because the authors relied on commercial selective media which could not accurately distinguish background organisms from the introduced test strains thus demonstrating the need for an alternative non GMO culture-based approach that can effectively discriminate target organisms from background contaminants during field studies.

Recently, Ongeng et al. (2011a) developed and validated a new culture method based on the use of spontaneous rifampicin-resistant mutants of non-virulent strains of *E. coli* O157:H7 and *S. typhimurium* and appropriate selective media. The method was successfully applied to study survival of *E. coli* O157:H7 and *S. typhimurium* in manure, manure-amended soil and in the manure-amended soil-plant ecosystem of cabbage under tropical field conditions in the Central Agro-Ecological zone of Uganda (Ongeng et al., 2011b,c,d). Whereas the rifampicin-based method seems to overcome the limitations of the culture methods mentioned before, it is still debatable whether the use of rifampicin-resistant mutants in field studies does not escalate the problem of antibiotic resistance in *Mycobacterium tuberculosis* considering that rifampicin is a common antibiotic used in the treatment of tuberculosis and that tuberculosis is still a huge public health problem in developing countries.

Fate of *E. coli* O157:H7 and *S. enterica* in manure and manure-amended soils

General observations

A number of studies have examined the fate of *E. coli* O157:H7 and *S. enterica* in manure and manure-amended soils worldwide. Tables 1 and 2 present summaries of those studies indicating the country of study, the strains and matrix used, applied experimental conditions, the reported survival times and microbiological methodology used. What is quite notable from Tables 1 and 2 is that much of the information on the behavior of *E. coli* O157:H7 and *S. enterica* in manure and manure-amended soil is based on studies directed towards the agricultural environment in temperate countries while very few studies exist that take into account conditions inherent to other climatic conditions, e.g., the tropical environment. In addition, a number of observations can be derived from studies presented in Tables 1 and 2 with regard to pathogen survival time: (i) in general, the reported survival times are inconsistent, which indeed reflects the variability of the considered environment and experimental methodologies used; (ii) persistence times of *E. coli* O157:H7 and *S. enterica* observed from studies conducted under field conditions are inconsistent with survival times that were determined in controlled environmental conditions in the laboratory, thus indicating that survival time determined under controlled environmental conditions cannot reliably be used to estimate survival under dynamic conditions typically encountered in the field; and (iii) the reported survival times are mostly based on experiments performed using inocula prepared at a single physiological age (e.g., harvested after 18 h of cultivation), yet in reality, *E. coli* O157:H7 and/or *S. enterica* when present in manure might be a composite of cells at various physiological ages.

Factors that influence the fate of *E. coli* O157:H7 and *S. enterica* in manure and manure-amended soils

The survival of *E. coli* O157:H7 and *S. enterica* in manure and/or manure-amended soil have been shown to depend on several factors. Summaries of reported studies on those factors, indicating the factor investigated, strains and matrix used, microbiological methodology applied, observed effects and the country of study are presented in Table 3. To enhance smooth flow of the discussion, we propose to group those factors into the following main categories and subsequently discuss them in detail: (i) physical and chemical characteristics of manure and soil; (ii) weather or atmospheric conditions; (iii) biological interactions; (iv) agricultural and livestock management practices; and (v) strain variation and physiological age of the cells.

Physical and chemical properties of manure and soil

The physical and chemical properties of importance include pH, oxygen availability, organic matter content and nutrient availability, natural antimicrobial substances, moisture, and texture and particle size distribution. The significance of pH in determining the fate of *E. coli* O157:H7 and *S. enterica* in agricultural matrices such as manure and manure-amended soil can be inferred from the fundamental impact pH is known to have on the physiology and metabolic activities of bacteria. Generally, a neutral pH is expected to favor survival whereas pH extremes should be detrimental to survival. However, this generalization may not be valid for *E. coli* O157:H7 and *S. enterica* in acidic pH conditions. *E. coli* O157:H7 and *S. enterica* while in the gastrointestinal tract of the animal host are exposed to acidic pH (Berg et al., 2004; Callaway et al., 2003) and the organisms seem to have developed molecular mechanisms to respond to the acid stress (Lin et al., 1996). On this basis therefore, the survival of *E. coli* O157:H7 and *S. enterica* in manure and manure-amended soil in the agricultural environment is expected to be favored by acid pH. This was observed to be the case for *E. coli* O157:H7 in cattle manure (Franz et al., 2005). On the other hand, alkaline pH is indeed detrimental to survival. Avery et al. (2009) showed that treatment of livestock wastes with lime at a rate of $10\,\mathrm{g\,L^{-1}}$ reduced *E. coli* O157:H7 counts in the matrix to a level which could not be detected by enrichment culture method. Similar results were also observed with *S. enterica* in other related studies (Bennett et al., 2003; Nyberg et al., 2011; Springfellow et al., 2010). In all cases, the pH of the lime treated matrices was all in the alkaline region. The detrimental effect of alkaline pH on survival of *S. enterica* in manure was also demonstrated in another study which revealed that treatment of bovine manure with 0.5% ammonia and 2% urea significantly reduced the decimal reduction time of the organism by 29.2 and 33.9 days at 4 °C and by 6.3 and 7.9 days at 14 °C, respectively (Ottoson et al., 2008). The manner in which alkalizing agent is used is important in determining survival. This was demonstrated in the work of Ottoson et al. (2008) which showed that a high manure to soil ratio in

Table 1. Overview of reported studies on survival of *E. coli* O157:H7 in manure and manure-amended soil indicating the country of study, the strains and matrix used, applied experimental conditions and the reported survival times.

Strains used	Matrix used	Experimental conditions	Reported survival time	Country of study	Reference
E. coli O157:H7 ATCC 43894 and ATCC 43888	Bovine manure	Laboratory setting, isothermal at 4, 23, 37, 45 and 70 °C	1 day–21 months	Russia	Kudva et al., 1998
E. coli O157:H7 ATCC 43894 and ATCC 43888	Bovine manure	Outdoor conditions	21 months	Russia	Kudva et al., 1998
Streptomycin-resistant *E. coli* O157:H7 NCTC 12900	Bovine manure	Laboratory setting, isothermal at 10 °C	>99 days	England	Bolton et al., 1999
Five strain mixture of GFP-labelled *E. coli* O157:H7	Bovine manure-amended soil	Laboratory setting, Isothermal at 5, 15 and 21 °C	77–231 days	USA	Jiang et al., 2002
E. coli O157 (strain 20001383)	Bovine manure	Field setting	16–64 days	England	Hutchison et al., 2004
E. coli O157 (strain 20001383)	Pig manure	Field setting	16 days	England	Hutchison et al., 2004
E. coli O157 (strain 20001383)	Sheep manure	Field setting	16 days	England	Hutchison et al., 2004
E. coli O157:H7 B6-914 *gfp*-91	Bovine manure-amended soil	Field setting	154–217 days	USA	Islam et al., 2004d
E. coli O157:H7 B6-914 *gfp*-91	Bovine manure-amended soil	Field setting	154–196 days	USA	Islam et al., 2005
E. coli O157:H7, unspecified strain	Bovine manure	Field setting	16–32 days	England	Hutchison et al., 2005
E. coli O157:H7, unspecified strain	Pig manure	Field setting	32 days	England	Hutchison et al., 2005
E. coli O157:H7, unspecified strain	Sheep manure	Field setting	63 days	England	Hutchison et al., 2005
E. coli O157:H7 B6-914 *gfp*-91	Bovine manure	Laboratory setting, isothermal at 20 °C	84–133 days	The Netherlands	Franz et al., 2005
E. coli O157:H7 MN626	Bovine manure	Field setting	92 days	USA	Mukherjee et al., 2006
E. coli O157:H7, unspecified strain	Bovine manure	Green house setting	8–12 weeks	Norway	Johannessen et al., 2005
E. coli O157:H7 B6-914 *gfp*-91	Bovine manure	Laboratory setting, isothermal at 10 °C in darkness; 80% moisture	85–123 days	The Netherlands	Franz et al., 2007a
Five *E. coli* O157:H7-pGFP(strains ATCC 43888, EO122K3995, K4492, and F4546)	Bovine manure-amended coil	Climate controlled growth chamber, 23 °C during day, 7 °C at night, 12-h photoperiod and light intensity of 600 to 700 mmol/m^2/s	60 days	USA	Zhang et al., 2009
E. coli O157:H7-Rifr (strain ATCC 43888)	Bovine manure, manure-amended soil	Screen house and field settings	4–12 weeks	Uganda	Ongeng et al., 2011b

Table 2. Overview of studies on survival of *Salmonella* spp. in manure and manure-amended soil indicating the country of study, the strains and matrix used, applied experimental conditions and the reported survival times.

Strains used	Matrix used	Experimental conditions	Reported survival time	Country of study	Reference
S. typhimurium DT104 (S8118/99)	Bovine manure	Field setting	34–120 days	England	Hutchison et al., 2004
S. typhimurium DT104 (S10570/99)	Pig manure	Field setting	56–120 days	England	Hutchison et al., 2004
S. Enteritidis PT4 (S8167/99)	Poultry manure	Field setting	56 days	England	Hutchison et al., 2004
S. typhimurium (χ^{3985} Δcrp-11 Δ cya-12)	Bovine manure-amended soil	Field setting	203–231 days	USA	Islam et al., 2004b
S. typhimurium (χ^{3985} Δcrp-11 Δ cya-12)	Compost-amended soil	Field setting	161–231 days	USA	Islam et al., 2004c
S. typhimurium, unspecified strain	Bovine manure	Field setting	32–42 days	England	Hutchison et al., 2005
S. typhimurium, unspecified strain	Pig manure	Field setting	16–32 days	England	Hutchison et al., 2005
S. typhimurium, unspecified strain	Sheep manure	Field setting	16 days	England	Hutchison et al., 2005
S. typhimurium, unspecified strain	Poultry manure	Field setting	63 days	England	Hutchison et al., 2005
S. typhimurium MAE119 –pGFP, *S. typhimurium* MAE110 -pGFP	Bovine manure-amended soil	Isothermal at 20 °C in darkness; 80% moisture	>133 days	The Netherlands	Franz et al., 2005
A multidrug-resistant and a drug-susceptible strain of *S. Newport*, unspecified strains	Cow manure	Isothermal at 25 °C	184 days	USA	You et al., 2006
A multidrug-resistant and a drug-susceptible strain of *S. Newport*, unspecified strains	Bovine manure-amended soil	Isothermal at 25 °C	405 days	USA	You et al., 2006
A multidrug-resistant and a drug-susceptible strain of *S. Newport*, unspecified strains	Bovine manure slurry	Storage in steel tanks	90 days	USA	Nicholson et al., 2005
A multidrug-resistant and a drug-susceptible strain of *S. Newport*, unspecified strains	Bovine manure-amended soil	Field setting	300 days	USA	Nicholson et al., 2005
Mixture of *S. typhimurium*, *S. Agona*, *S. Hadar*, *S. Oranienburg*, unspecified strains	Hog manure slurry	Isothermal at 4, 25 and 37 °C	>300 days at 4 °C	Canada	Arrus et al., 2006
S. typhimurium LT2, virulence attenuated	Bovine manure and manure-amended soil	Screen house and field settings	6–14 weeks	Uganda	Ongeng et al., 2011b

Table 3. Summary of reported studies on the factors that affect the survival of *E. coli* O157:H7 and *Salmonella* spp. in manure and/or manure-amended soil, indicating the factor investigated, strains and matrix used, observed effects and the country of study.

Factor investigated	Strains used	Matrix used	Experimental conditions	Observed effects	Country of study	Reference
Background microbial community	*E. coli* O157:H7 ATCC 43894 (Stx1^{+} Stx2^{+}) and ATCC 43888 (Stx1^{-} Stx2^{-})	Bovine manure	Laboratory setting, isothermal at 4, 23, 37, 45 and 70 °C	Background microbial community enhanced the decline rate	Russia	Kudva et al., 1998
Background microbial community	*E. coli* O157:H7 and *S. typhimurium*, unspecified strains	Bovine manure	Laboratory setting, isothermal at 4, 20, and 37	Background microbial community enhanced the decline rate	USA	Himathongkham et al., 1999a
Background microbial community	Five strain mixture of GFP-labelled *E. coli* O157:H7	Bovine manure-amended soil	Laboratory setting, isothermal at 5, 15 and 21 °C	Background microbial community enhanced the decline rate	USA	Jiang et al., 2002
Background microbial community	A mixture of a multidrug-resistant and a drug-susceptible *S. Newport*, unspecified strains	Bovine manure and manure-amended soil	Laboratory setting, isothermal at 25 °C	Background microbial community enhanced the decline rate	USA	You et al., 2006
Background microbial community	*E. coli* O157:H7 B6-914 *gfp*-91	Bovine manure	Predicted by COLLIWAVE simulation model	Background microbial community enhanced the decline rate	The Netherlands	Semenov et al., 2010
Background microbial community	*E. coli* O157:H7 B6-914 *gfp*-91, *typhimurium* MAE119 (Δ*agf*D 101 *saw*), MAE110 (p*agf*D1 *rdr*)	Bovine manure and manure-amended soil	Laboratory setting, isothermal at 20 °C, 80% moisture and in the dark	Faster decline in manure from pure straw diet than in manure from grass silage and maize silage	The Netherlands	Franz et al., 2005
Physical and chemical properties of the soil	*E. coli* O157:H7, unspecified strain	Soil	Laboratory setting, isothermal at 25 °C	Mortality rate was higher in coarse-loamy-mixed-active-mesic-Fluventic-Dystrudept than in fine-silty, -mixed, active, -mesic-Oxyaquic-Fragiudalf soil	England	Mubiru et al., 2000
Soil management type	*E. coli* O157:H7 B6-914 *gfp*-91, Two *S. typhimurium* (MAE119: Δ*agf*D 101 *saw*; MAE110: p*agf*D1 *rdr*)	Bovine manure-amended soil	Laboratory setting, isothermal at 20 °C, 80% moisture and in the dark	Faster decline in organically managed soil than in conventionally managed soil	The Netherlands	Franz et al., 2005
Livestock management system	*E. coli* O157:H7 B6-914 *gfp*-9	Bovine manure	Laboratory setting, isothermal at 10 °C, 80% humidity, 80% moisture	Survival was longer in manure from organically managed livestock than in manure from conventionally managed livestock	The Netherlands	Franz et al., 2005
Aeration	*E. coli* O157:H7 ATCC 43894 and ATCC 43888	Ovine manure and slurry	Manure pile exposed to outdoor conditions	Aeration enhanced the decline rate	Russia	Kudva et al., 1998
Aeration	*S. typhimurium,* unspecified strain	Bovine and pig manure	Manure heaps kept in farm house	Aeration enhanced survival in pig manure but had no effect on survival in bovine manure	England	Nicholson et al., 2005
Rhizosphere	*E. coli* O157:H7 B6914-*gfp*	Bovine manure-amended soil	Climate controlled growth chamber (25 °C, 80% relative humidity, 12 h light cycle),	Persistence was enhanced by 100-300% in rhizosphere of rye compared to bulk soil	USA	Gagliardi & Krans, 2000
Oxygen availability	*E. coli* O157:H7 B6-914 *gfp*-91	Bovine manure	Predicted by COLIWAVE simulation model		The Netherlands	Semenov et al., 2010

(*continued*)

Table 3. Continued

Factor investigated	Strains used	Matrix used	Experimental conditions	Observed effects	Country of study	Reference
				Survival was more under anaerobic followed by semi-aerobic and least under aerobic conditions		
Temperature	*E. coli* O157:H7, unspecified strain	Bovine manure-amended soil	Laboratory setting, isothermal at 5, 15 and 21 °C	Survival time increased with temperature	USA	Jiang et al., 2002
Temperature	*E. coli* O157: H7 and *S. typhimurium*, unspecified strains	Bovine manure	Laboratory setting, isothermal at 4, 20, and 37	Decline rate increased with temperature	USA	Himathongkham et al., 1999a
Temperature	*E. coli* O157: H7, unspecified strains	Bovine manure	Laboratory setting, isothermal at 5, 22 and 37 °C	Decline rate increased with temperature	USA	Wang et al., 1996
Temperature	*E. coli* O157:H7 ATCC 43894 and ATCC 43888	Bovine manure	Laboratory setting, isothermal at 4, 23, 37, 45 and 70 °C	Decline rate increased with temperature	Russia	Kudva et al., 1998
Temperature	Cocktail mixture containing *S. typhimurium*, *S. Agona*, *S. Hadar*, *S. Oranienburg*	Hog manure slurry	Laboratory setting, isothermal at 4, 25 and 37 °C	Survival time reduced with temperature	Canada	Arrus et al., 2006
Temperature	*E. coli* O157:H7 MN626	Bovine-manure amended soil	Laboratory setting, isothermal at 4 °C and at ambient conditions	Decline rate was faster at 4 °C than at ambient temperatures	USA	Mukherjee et al., 2006
Temperature	*E. coli* O157:H7 B6-914 *gfp*-91, *S. typhimurium typhimurium* MAE119-*gfp*	Bovine manure	Laboratory setting, isothermal at 7, 16, 23 and 33 °C, and dynamic with daily oscillations with amplitudes of 4 or 7 °C	Survival time reduced with increasing mean temperatures and with increasing amplitude in daily temperature oscillations.	The Netherlands	Semenov et al., 2007
Water activity (a_w)	*S. Enteritidis*, *S. typhimurium*	Chicken manure	Isothermal at 20 °C	Decline rate highest at intermediate aw (0.89) than at levels above or below it.	USA	Himathongkham et al., 1999b
Moisture condition	*E. coli* O157:H7	Bovine manure-amended soil	Laboratory setting, isothermal at 20 °C	*E. coli* O157:H7 grew and persisted at all but the lowest moisture levels examined, manure content modulated the effect of water on growth	USA	Berry & Miller, 2005
Moisture condition	*E. coli* O157:H7 ATCC 43888 and *S. typhimurium* LT2	Bovine manure, manure-amended soil	Screen house setting, moisture controlled, fluctuating temperature	High moisture level($\geq$80 %RH) enhanced persistence and decline of 4 log and 7 log CFU/g inoculum, respectively	Uganda	Ongeng et al., 2011b
Moisture condition	*E. coli* O157:H7 ATCC 43888 and *S. typhimurium* LT2	Bovine manure, manure-amended soil	Field setting	Moisture condition had no effect on survival irrespective of inoculum level	Uganda	Ongeng et al., 2011b
Rhizosphere	*E. coli* O157:H7 ATCC 43888 and *S. typhimurium* LT2	Bovine manure-amended soil	Field setting with cabbage	Rhizosphere of cabbage plants enhanced survival of the 7 log CFU/g inocula but had no on survival of the 4 log CFU/g inocula	Uganda	Ongeng et al., 2011d
pH	*E. coli* O157:H7	Bovine manure	Laboratory setting, isothermal at 20 °C	Lime treatment significantly reduced *E. coli* O157:H7	USA	Avery et al., 2009

combination with a higher incubation temperature rapidly neutralized the pH of the matrix and thus increased the risk potential for *Salmonella* re-growth.

Moisture is an important physiological requirement for microbial growth and survival. Its functionality in determining microbial processes is normally measured in terms of the minimal water activity (a_w) for a particular process to take place. The influence of moisture on survival of human enteric pathogenic bacteria such as *E. coli* O157:H7 and *S. enterica* in manure and/or manure-amended soil seems to depend on the concentration of the pathogen in the matrix. Ongeng et al. (2011b) compared the survival of *E. coli* O157:H7 and *S. typhimurium* in manure and manure-amended soil between matrices maintained at high moisture level (80% RH) and those left to desiccate over time. The authors observed that maintaining the matrices at high moisture level enhanced survival of the 7 log CFU/g inocula, but high moisture level was detrimental to survival of the 4 log CFU/g inocula. In the same study, it was observed that *S. typhimurium* survived significantly longer than *E. coli* O157:H7 in the desiccated manure and manure amended soil matrices thus suggesting that *S. enterica* might be capable of withstanding water stress much better than *E. coli* O157:H7. Molecular studies could therefore be performed to delineate the fundamentals behind the differential response of *S. enterica* and *E. coli* O157:H7 to moisture stress in the agricultural environment. Furthermore, it appears there is a narrow region of a_w within which survival of enteric pathogenic bacteria in agricultural matrices such as manure and manure-amended soil is severely constrained. This scenario has been observed in the case of *Salmonella* in chicken manure (Himathongkham et al., 1999b). The authors showed that the destruction of *S. enteritidis* and *S. typhimurium* in manure held at 20 °C was most rapid at an intermediate a_w level around 0.89 compared to inactivation at a_w above or below that level. In particular, the authors showed that as a_w decreased below 0.89 the die off of *Salmonella* became slower compared to the die off at a_w levels above it. In fact the time needed for a million-fold reduction under extreme dry conditions was projected to be more than 3 months as compared to 8 days at an a_w of 0.89 and 30 days at a_w above 0.89.

Oxygen is an important physiological factor that affect energy metabolism in bacteria and as such can have a significant impact on survival capabilities of *E. coli* O157:H7 and *S. enterica* in agricultural matrices such as manure and manure-amended soil. Kudva et al. (1998) observed that *E. coli* O157:H7 survived significantly longer in non-aerated bovine manure than in aerated samples by more than 6 months. A similar result was also obtained in another study where survival of *E. coli* O157:H7 was longer in unturned manure heaps than in turned manure heaps by approximately 9 weeks (Fremaux et al., 2008). The difference in survival was presumptively attributed to the greater availability of oxygen in aerated and turned manure than in non-aerated and unturned manure, respectively. This suggested that *E. coli* O157:H7 survives better under anaerobic than in aerobic condition. The positive impact of anaerobiosis on survival of *E. coli* O157:H7 was conclusively demonstrated by Semenov et al. (2011). The authors compared survival of *E. coli* O157:H7 in cattle manure and slurry between a controlled anaerobic set-up and a controlled aerobic set-up and observed that the organism persisted under anaerobic and aerobic condition by approximately 2 weeks and 6 months, respectively. On the other hand, oxygen availability does not seem to affect the survival of *S. enterica*. This scenario is well illustrated in the work of Semenov et al. (2011), where it was demonstrated that survival of *S. typhimurium* in manure and slurry between an anaerobic set-up and an aerobic set-up was identical. *E. coli* O157:H7 and *S. enterica* are both facultative anaerobic organisms. In the presence of oxygen, the organisms undergo respiratory metabolism, but in the absence of oxygen they switch to fermentative metabolism. It is therefore quite surprising that in livestock wastes, oxygen is detrimental to survival of *E. coli* O157:H7 but has no effect on survival of *S. enterica*. More work is therefore needed to elucidate on the differential effects of oxygen on survival of *E. coli* O157:H7 and *S. enterica* in livestock wastes. Nevertheless, in the case of *E. coli* O157:H7 it would be of interest that anaerobic storage of manure or conditions that lead to anaerobiosis during storage should be avoided.

The chemoheterotrophic nature of *E. coli* O157:H7 and *S. enterica* dictates that their survival in manure and/or manure-amended soil critically depends on nutrient availability. By inference, therefore, manure and/or soil with readily available carbon and nitrogen sources as well as other nutrients are expected to support survival and/or growth of the organisms better than matrices with poor nutrient endowment. This assertion is supported by the work of Franz et al. (2008), which demonstrated a positive correlation between dissolved organic carbon or dissolved organic nitrogen and survival of *E. coli* O157:H7 manure-amended soils. The quality of organic matter as well as the carbon to nitrogen ratio (C/N) of the matrix does affect survival. Manure and manure-amended soils with less degradable organic matter and high C/N ratio release nutrients at a much slower rate than those with readily degradable organic matter and balanced C/N ratio. Thus *E. coli* O157:H7 and *S. enterica* are expected to decline faster in matrices with the former than in those with the latter characteristics. Indeed, this was shown to be so by the results of Franz et al. (2008), which demonstrated that *E. coli* O157:H7 survived longer in soils amended with cattle manure slurry than in soils amended with solid manure. Therefore, as observed by Franz & van Bruggen (2008), manure amendment strategies that lead to oligotrophic condition in the soil (e.g., application of solid manure instead of slurry) might provide a plausible means of limiting the persistence of enteric pathogens such as *E. coli* O157:H7 and *S. enterica* in the agricultural environment.

Several natural antimicrobial substances are likely to be present in manure and subsequently in the soil following manure amendment. Of interest are the natural antimicrobials that find their way into the manure from the feed. Plant carboxylic phenols (tannins) are the best probably known. Whereas research on antimicrobial effect of tannins has mainly been focused on rumen bacteria (Nelson et al., 1998 and references therein), investigations involving manure-borne human enteric bacterial pathogens in the outside environment have started to emerge. Using an in vitro experimental set-up, Min et al. (2007) showed that chestnut and mimosa tannins reduced the growth rate of *E. coli* O157:H7 in a model medium and that the degree of growth reduction increased with tannin concentration. In another study, Wells et al. (2005) investigated the effect of common phenolic acids on viability of *E. coli* O157:H7 in cattle feces and observed that the death rate of the organism increased by 3 to 23- fold depending on the concentration and nature of the phenolic compound. These results suggest that inclusion of tanniferous forage plants in the diet of the animal may reduce the length of time that manure-borne enteric pathogens such as *E. coli* O157:H7 and *S. enterica* can survive when contaminated manure is incorporated into the soil. Thus a well-designed experiment comparing survival of *E. coli* O157:H7 and/or *S. enterica* in manure from animals fed tanniferous forages with those on base feed should provide empirical evidence to that effect.

Following the application of contaminated manure or manure slurry to top soil, a significant fraction of the pathogen population can percolate to deeper layers of the soil and possibly to ground water level. An elegant study performed by Bech et al. (2010) demonstrated that *S. typhimurium* could leach down to a 1 m depth in loamy soil at concentrations reaching 1.3×10^5 CFU/ml of leachate. However, in a typical soil survival experiment performed in the field, the leached fraction of the test population is never taken into account or unknowingly considered to be dead, which indeed lead to underestimation of survivor counts. Factors which affect porosity as well as adsorption and filtration properties of the soil such as texture and particle size distribution within the soil can influence the extent to which pathogens can leach through the soil matrix. Variation in texture and particle size distribution is mainly manifested in differences between soil types and differences between intact and disturbed soils. Bech et al. (2010) compared leaching potential of *S. typhimurium* between loamy and sandy soil monoliths amended with manure slurry and observed that leaching of the organism through the matrix was more pronounced in loamy than in sandy monoliths. In fact the concentration of *S. typhimurium* in the leachate from loamy soil during the 27 days of the experiment ranged from 300 to 1.3×10^5 cells/ml while the organism was only sporadically detected at low levels (<20 cells/ml) in the leachate from sandy soil. Gagliardi & Krans (2000) used a simulated rainfall experiment to investigate the effect of soil type (sandy loam, clay loam and sandy loam) and tillage (disturbed versus intact soil core) on the ability of *E. coli* O157:H7 to leach through soil matrix following manure application. Results showed that *E. coli* O157:H7 leached though all the soil types investigated except in intact clay loam core where leaching ceased just after three days due to clogging. The most interesting observation from this study was that in situations where leaching occurred throughout the experimental period, the concentration of *E. coli* O157:H7 in the leachate was much higher (0.64 to 30.97 times) than the number initially introduced into the matrix thus indicating growth of the organism in the leachate. Considering the importance of leaching as described above, it would therefore be prudent that a strategy is designed to correct for the effect of leaching in soil survival experiments performed in real agricultural fields.

Weather or atmospheric conditions

Atmospheric variables considered here are temperature, solar radiation and moisture. Of all the three variables the effect of temperature is the most extensively studied. Temperature has received so much attention probably because of the profound effect it has on growth and decay rates of bacteria generally. Review of literature revealed that most of the experimental work that investigated temperature effects focused on static temperature set-ups (Table 3). Under isothermal temperature conditions, the decline rates of *E. coli* O157:H7 and/or *S. enterica* in manure and/or manure-amended soil have been shown to increase with temperature (Arrus et al., 2006; Himathongkham et al., 1999a; Kudva et al., 1998; Semenov et al., 2007; Semenov et al., 2010; Wang et al., 1996) although results of a few studies indicate the opposite (Bach et al., 2005; Jiang et al., 2002; Mukherjee et al., 2006). The response of *E. coli* O157:H7 and/*S. enterica* to static temperature appears also to depend on temperature range and the interval within the range under consideration. Ongeng et al. (2011e) showed that the decline rate of *S. typhimurium* in manure and manure-amended soil increased with temperature between 16 and 25 °C, stagnated between 25 and 37 °C and increased again between 37 and 42 °C. Notwithstanding the possible influence of other factors inherent in matrices used in different studies, it becomes quite apparent that no general conclusion can be drawn on how static temperature affects survival in manure and/or manure-amended soil. To date, the only controlled study that examined the effect of fluctuating temperature on survival was performed by Semenov et al. (2007). The authors showed that the survival of *E. coli* O157:H7 and *S. typhimurium* in bovine manure reduced with increasing amplitude in daily temperature oscillations. An important inference that can be drawn from such a finding is that, since daily temperature regimes vary among different geographical regions, the survival of *S. enterica* and/or *E. coli* O157:H7 in manure and manure-amended soil is expected to be different among climatically different geographical regions.

The extent to which enteric bacterial pathogens such as *E. coli* O157:H7 and *S. enterica* can survive in manure or manure-amended soil inevitably depends on the degree of exposure to solar radiation. Meays et al. (2005) examined the effect of solar radiation using generic *E. coli* as a model organism in a faecal-pat experiment set-up with 0, 40, 80 or 100% shade cloth and observed that the decline rate of the organism in the faecal pats decreased with the degree of shading. From a practical perspective, the results of Meays et al. (2005) suggest that manure-borne pathogens are likely to decline faster in the tropics than in the temperate regions due to the high intensity of solar radiation in the former than in the latter geographical region. In another study, Hutchison et al. (2004) investigated using field experimental set-up, the effect of length of time before soil incorporation of livestock wastes spread to land on the rate of decline of zoonotic agents including *E. coli* O157:H7 and *S. enterica*. Their results showed that bacterial decline was significantly more rapid when the wastes were left on the soil surface compared to when the wastes were immediately incorporated into the soil. Based on this finding, it is apparent that delaying the incorporation of contaminated manure into the soil can reduce the length of time that manure-borne pathogens remain viable in the soil. However, from environmental safety perspective, spreading contaminated manure on the soil surface in the field raises the question as to whether such a practice may not exacerbate the spread of the pathogens in the agricultural environment instead. This is particularly important because of the scavenging activities of insects, vermin, birds and other wild animals in agricultural fields (Adesiyun et al., 1998; Clough et al., 2003; Kapperud et al., 1998), and the occurrence of heavy rainfall that can cause surface runoff of contaminated manure directly into watercourses (Chalmers et al., 2000; Maule, 2000).

The effect of moisture on survival of *E. coli* O157:H7 and *S. enteric*a in manure and manure-amended soil has been discussed in section 3.2.1 of this review. However, it is important to note that moisture level in manure and/or manure-amended soil depends on humidity in the environment in question. Under field conditions, a key factor influencing humidity is the level of precipitation and rainfall pattern. This is clearly illustrated by results of Ongeng et al. (2011b) which show that moisture condition affected survival of *E. coli* O157:H7 and *S. typhimurium* in manure and manure-amended soil in a screen house experiment but not in the field. Variation in rainfall pattern has a dramatic effect on decline pattern of enteric bacterial pathogens in manure and manure-amended soil due to the dehydration-rehydration phenomenon (Ongeng et al., 2011b).

Biological interactions

The composition and density of background microbial community greatly affect the survival of *E. coli* O157:H7 and *S. enterica* in manure and manure-amended soil ecosystems. Two key findings provide credence to this effect: (i) the work of Jiang et al. (2002) which demonstrates that *E. coli* O157:H7 declined more rapidly in non-sterilized soils amended with manure at a ratio of one part manure to ten parts soil than in soil samples containing dilute amounts of manure; and (2) the results of You et al. (2006) which show that *S. Newport* persisted longer in sterilized manure-amended soil than in non-sterilized manure-amended soil by up to 73 days. Interestingly, *E. coli* O157:H7 and *S. enterica* seem to survive better in manure-amended soil than in manure (Jiang et al., 2002; Ongeng et al., 2011b; You et al., 2006). This implies that manure-amended soil provides more favorable conditions for survival despite the fact that manure is richer in nutrients than soil. A probable reason for this phenomenon, as suggested by Ongeng et al. (2011b), could be that indigenous organisms in manure, e.g. coliforms, exert more antagonistic effects to *E. coli* O157:H7 and *S. enterica* than indigenous soil flora do. This suggestion is in consonant with the work of Franz et al. (2007b), which show that the overall survival time of *E. coli* O157:H7 in cattle manure was negatively correlated with the number of coliforms. Considering that coliforms normally occur in high numbers in cattle feces as opposed to soil (Cox et al., 2005), it has been argued that the faster decline of *E. coli* O157:H7 and *S. enterica* consistently observed in manure compared to manure-amended soil can partly be attributed to the dilution effect of the soil fraction on the density of coliforms and other indigenous organisms present in manure (Ongeng et al., 2011b). An important conclusion that can be drawn from the aforementioned observations is that amendment of fresh manure to soil significantly enhances the survival of *E. coli* O157:H7 and *S. enterica* in the agricultural environment.

In complex environmental substrates such as manure and manure-amended soil, biological interactions impacting on survival of enteric pathogens such as *E. coli* O157:H7 and *S. enterica* inevitably involve several entities including viruses, bacteria, fungi and protists. However, only the effects of protists have so far been explicitly studied using protozoa. Information available to-date indicates that the presence of protozoa can either enhance or reduce survival. Brandl et al. (2005) showed that the protozoan ciliate *Tetrahymena* enhanced survival of *S.* Thompson by concentrating the pathogen in the protozoal feeding vesicles and subsequently releasing vesicles containing high numbers of the pathogen into the outside environment. A similar result was also obtained in another study conducted with *E. coli* O157:H7 and the protozoan *Acanthamoeba polyphaga* (Barker et al., 1999). The negative impact of protozoan grazing on survival was on the other hand demonstrated in a study where reduction in population size of *S. typhimurium* CFU levels in soil was associated with growth of the protozoan (Garcìa et al., 2010). This finding concurs with the results of Puri & Dudley (2010) which show that protist communities played a major role in reducing *E. coli* O157:H7 in bovine manure compost.

Agricultural management practices

Agricultural and livestock management practices vary a lot. The variation undoubtedly affects the biological and physico-chemical properties of manure and the soil and hence survival of the pathogen. In this review, we shall concentrate on differences that have been observed between organic and conventional production systems, and on the effect of animal feeding regimen on survival. Franz et al. (2007a) compared the survival of *E. coli* O157:H7 in manure samples between organic and low in-put conventional dairy farms and observed that the organism survived longer in manure from organic farms than in manure samples from low in-put conventional farms. The difference in survival between the two manure sources was partly attributed to the higher coliform content of the organic manure compared to low in-put manure. In another study, Franz et al. (2005) showed that *E. coli* O157:H7 survived longer in manure-amended soil with soil samples from conventionally managed fields than with soil samples from organically managed fields. A similar result was also later reported by Semenov et al. (2008). These findings therefore provide sufficient evidence to conclude that manure-borne enteric bacteria survive better in manure and manure-amended soil under conventional farming system than under organic system; thus suggesting that contamination of fresh vegetables with manure-borne enteric pathogenic bacteria is most likely to occur under the former than in the later production system. In fact, fruits and vegetables from conventional production systems have been identified as a major source of food-borne diseases than those from organic systems (Leifert et al., 2008 and references therein).

Animal feeding regimen has a strong influence on manure composition and hence survival (Bach et al., 2005; Franz et al., 2005). Franz et al. (2005) showed that *E. coli* O157:H7 and *S. typhimurium* declined significantly faster in manure derived from straw diet than in manure from the diet of grass silage plus maize silage. The difference in decline rate was attributed to the higher pH and higher fiber contents of manure from former diet than manure from the latter diet. The negative effect of high fiber was also illustrated in a feed evaluation study where it was shown that increasing the fiber content of a concentrate diet from 10 to 20% reduced fecal shedding of *E. coli* O157:H7 orally inoculated to lambs (Lema et al., 2002). The influence of animal diet on survival is further exemplified by the results of Bach et al. (2005) which show that *E. coli* O157:H7 survived longer in feces of steers fed corn than in feces of steers fed barley under isothermal condition at 4 °C. This observation is a consequence of the fact that barley-fed cattle have a higher fecal pH and lower volatile fatty acid concentration compared with corn-fed animals (Berg et al., 2004; Buchko et al., 2000). A practical implication of these findings can be viewed in terms of differences in animal feeding regimen between different geographical regions. For instance, in some tropical regions such as in Sub-Saharan Africa, cattle on small-holder farms feed exclusively on grass or straw which is high in lignin and fiber which is in contrast with feeding regimens of cattle in developed temperate countries where concentrate is supplemented to the base feed. On this basis, it becomes clear that the survival of *E. coli* O157:H7 and/or *S. enterica* in manure from cattle fed on low quality diet such as is the case on small-holder farms in Sub-Saharan Africa will indeed be different from survival times determined using manure derived from cattle fed on high quality diet in developed temperate countries. This scenario is well reported in Ongeng et al. (2011b).

Strain variation and physiological age of the cells

The bulk of information available on the fate of *E. coli* O157:H7 and *S. enterica* in manure, manure-amended soil and in the manure-amended soil plant ecosystem of fresh vegetable crops are based on experiments performed with single strains or a composite of a few different strains (Table 1). However, reports on studies that make direct comparison between strains on the fate of the two organisms in the agricultural environment are very scanty. It was only recently that Franz et al. (2011) compared the survival capability of 18 strains of *E. coli* O157 (8 animals, 1 food and 9 human isolates) in cattle manure. In this study, it was shown that survival time of *E. coli* O157 was significantly higher for human isolates than for the animal isolates. Principal component analysis then revealed that the long-surviving strains were capable of oxidising organic acids (propionic acid, α-ketobutyric acid and α-hydroxybutyric acid) much faster than the short-surviving strains. Physiological age of cells undoubtedly affects the behavior of microbial cells. This has been shown to be the case in a number of studies in other subject areas such as bioremediation (Cunliffe et al., 2006 and references therein), antimicrobial resistance (Luidalepp, 2011) and thermobacteriology (Martínez et al., 2003 and references therein). However, there is no information on the effect of physiological age of cells and/or inoculum heterogeneity on the behavior of *E. coli* O157:H7 and *S. enterica* in the manure-amended soil-plant ecosystem of vegetable crops. This is a potential subject area for research.

Interrelationships between factors that affect survival

The effects of factors discussed in the previous sections were mostly studied in isolation. However, in reality, these factors interact to determine survival. This has an implication for the application of research results in produce safety management. For instance, moisture is necessary for bacterial survival, but moisture retention in the soil depends on soil organic matter level which also influences soil microbial community structure. This means that the survival of *E. coli* O157:H7 and/or *S. enterica* in highly weathered soils such as those found in Sub-Saharan Africa will unlikely be the same as in fertile soils of temperate countries. Another interesting correlation exists between geographical location, environmental temperature, animal diet and feeding regimen. Whereas temperature has a dramatic effect on survival, temperature regime varies with geographical location and geographical location has a significant effect on feed quality which affects manure composition. Ongeng et al. (2011b) compared survival times of *E. coli* O157:H7 and *S. typhimurium* in manure and manure-amended soil obtained from experiments conducted under tropical field conditions in the Central Agro-Ecological Zone of Uganda with survival times obtained from experiments conducted in several temperate countries. The authors found that persistence times of the organisms under tropical conditions were much shorter than earlier observed under temperate conditions, which implies that biophysical conditions in the tropics might be more detrimental to *E. coli* O157:H7 and *S. typhimurium* than is the case in the temperate environment. In general, it can therefore be stated that due to interrelationships that exist in terms of the nature and levels of abiotic, biotic, edaphic and climatic factors that affect survival of *E. coli* O157:H7 and *S. enterica* in manure and manure-amended soil between geographical regions, the fate of such enteric pathogens should be determined under specific agricultural environment where vegetable cultivation takes place.

Fate of *E. coli* O157:H7 and *S. enterica* in the soil-plant ecosystem of fresh vegetable crops

Upon recognizing that use of non-composted livestock manure to fertilize agricultural soils or application of contaminated waste water to irrigate crops can introduce manure-borne human pathogens directly into the soil and subsequently leading to contamination of fresh vegetables during cultivation, a number of studies have examined the fate of *E. coli* O157:H7 and/or *S. enterica* in the soil-plant ecosystem of vegetable crops. A summary of those studies indicating the bacterial strains and vegetable used in the investigation, microbiological methodology applied, results obtained and the country of study is presented in Tables 4 and 5. An important observation that can be made from those tables is that the bulk of information on the behavior of *E. coli* O157:H7 and *S. enterica* in the soil-plant ecosystem of fresh vegetable crops is derived from studies performed in temperate or simulated temperate environment, but information from other climatic conditions is very scarce. Considering that large volumes of fresh produce traded on international market originate from diverse climatic regions it becomes prudent that more work should be done to understand the behavior of the pathogens in the soil-plant ecosystem in other climatic environments. The soil-plant ecosystem provides manure-borne bacterial pathogens such as *E. coli* O157:H7 and *S. enterica* with rather unique ecological niches different from those encountered in the manure and manure-amended soil ecosystems. These niches include the rhizosphere, the phyllosphere and the endosphere of plant tissues. Survival of the pathogens in the soil-plant ecosystem therefore depends on their ability to withstand both the biotic and abiotic stresses likely to be encountered in those niches.

The rhizosphere is a nutrient-rich ecological site where competition between microbial communities is known to be fiercely high (Berg et al., 2005). Experimental results available to date indicate that *E. coli* O157:H7 and *S. enterica* survive better in the rhizosphere of vegetable plants than in bulk soil (Ibekwe et al., 2004, 2006; Ongeng et al., 2011d; Semenov et al., 2009) or the phyllosphere (Ibekwe et al., 2009; Islam et al., 2004a). The fundamental bases for the enhanced survival of *E. coli* O157:H7 and *S. enterica* in the rhizosphere has not yet been delineated. However, in general, microbial survival in the rhizosphere has been associated with the ability of an organism to produce antibiotics or to withstand antibiotic activity. Studies with other bacteria have shown that organisms which had antifungal and/or antibacterial activity were greatly enriched in the rhizosphere of different plants (Berg et al., 2002, 2005). Rhizosphere competence has also been linked to the ability of colonizing bacteria to use root exudates as sources of carbon, and to synthesise compatible solutes to counter osmolarity dynamics in the rhizosphere (Miller & Wood, 1996). The constant supply of nutrients by root exudates is therefore thought to favor colonization and enhanced persistence of bacteria in the rhizosphere compared to bulk soil (Yang et al., 2001). In addition, as suggested by Ongeng et al. (2011d), enhanced survival in the rhizosphere could also be due to better moisture maintenance in the soil due to canopy provided by the plants. On the other hand, rhizosphere effect on survival of manure-borne enteric bacteria such as *E. coli* O157:H7 and *S. enterica* seems to depend on inoculum density. This was shown to be the case for *E. coli* O157:H7 and *S. typhimurium* in a study which demonstrated that the rhizosphere of cabbage plants enhanced survival of the organisms in manure-amended soil at inoculum concentration of 7 log CFU/g, but not at 4 log CFU/g under tropical field conditions (Ongeng et al., 2011d). Another important outcome from this study was that an enhanced persistence of the 7 log CFU/g inocula was associated with long-term contamination of cabbage plants, which occurred till harvest. Such an observation has a far reaching ramification for the safety of the vegetable because of the likely possibility of vegetables being contaminated with pathogens that have acquired antibiotic resistant genes from the rhizosphere. This argument is based on the fact that the rhizosphere is known to be a hot spot for gene exchange (Knudsen et al., 1988). For instance, conjugative transfer of chromosomal genes from the biocontrol strain *Pseudomonas fluorescens* CHA0 to the clinical strain *Pseudomonas aeruginosa* PAO1 in the rhizosphere of wheat in contrast to bulk soil was demonstrated by Troxler et al. (1997). Genetic experiments could therefore be conducted to explore the occurrence of antibiotic resistance genes in the rhizosphere of fresh vegetable plants and to determine whether enteric pathogenic bacteria such as *E. coli* O157:H7 and *S. enterica* can indeed acquire such resistance genes from the rhizosphere.

Escherichia coli O157:H7 and *S. enterica* are capable of invading and surviving endophytically in plant tissues. This scenario has mostly been observed with seedlings of temperate crops in controlled environmental conditions and data are available for a few vegetables including lettuce (Franz et al., 2007b; Johannessen et al., 2005; Jablasone et al., 2005; Solomon et al., 2002), cress (Jablasone et al., 2005), tomatoes (Guo et al., 2001, 2002), mung bean sprouts (Warriner et al., 2003b), radish sprouts (Itoh et al., 1998; Jablasone et al., 2005) and alfalfa sprouts (Dong et al., 2003). Published reports on the occurrence of internalized *E. coli* O157:H7 and/or *S. enterica* in mature plants has been very scarce. It was only until recently that the phenomenon was demonstrated with cabbage cultivated under tropical field conditions in the Central Agro-Ecological Zone of Uganda (Ongeng et al., 2011c,d). In these studies, the authors showed that *E. coli* O157:H7 and *S. typhimurium* survived endophytically in cabbage leaves throughout cultivation till harvest when introduced into the soil at 7 log CFU/g but not at 4 log CFU/g. This observation implicitly indicates that the inoculum size is an important factor that influences the internalization phenomenon.

In addition, the likelihood of internalization of enteric pathogenic bacteria in vegetable tissues seems to depend on plant type (Jablasone et al., 2005), plant age (Ongeng et al., 2011c) and the presence of insects (Erickson et al., 2010c). Jablasone et al. (2005) showed that *S. typhimurium* became internalized in lettuce and radish but not in spinach seedlings under the same experimental conditions, thus suggesting the possible influence of the plant genotype. Fundamental research to identify genotypic factors that restrict entry of enteric pathogens into plant tissues could be explored. Such results could then be incorporated into plant breeding programmes to improve vegetable safety. With respect to plant age, results of Ongeng et al. (2011c) indicate that *E. coli* O157:H7 and *S. typhimurium* got internalized in cabbage leaf tissues and survived endophytically in the plant till harvest when inocula were introduced into the soil at the point of transplantation. However, the authors observed no internalization phenomenon when the organisms were introduced into the soil 56 or 105 days post-transplantation, thus suggesting that mature plants could have already developed protective mechanisms to deter pathogen access to internal tissues of the plant. The long-term survival of *E. coli* O157:H7 and *S. typhimurium* in internal tissues of cabbage as observed in

Table 4. Overview of reported studies on survival of *E. coli* O157:H7 in the manure-amended soil-plant ecosystem indicating the bacterial strains and vegetable used in the investigation, microbiological methodology applied experimental conditions, results obtained and the country of study.

Strains used	Vegetable used	Experimental conditions	Detection method	Results obtained	Country of study	Reference
E. coli O157:H7 ATCC 43895-pGFP	Lettuce	Laboratory set-up: 20 °C, 24 h illumination cycle	Gfp coupled with microscopy and plate counting on selective medium	*E. coli* O157:H7 entered into lettuce plant through the root system and migrated throughout the edible portion of the plant	USA	Solomon et al., 2002
A cocktail mixture of *E. coli* O157:H7 strains ATCC 700728 and ATCC 43888	Spinach	Field set-up or Green house	Plate counting on selective medium	Water availability significantly affected survival	USA	Gutiérrez-Rodrìguez et al., 2011
E. coli O157:H7 NCTC 1200, non-toxigenic strain	Lettuce	Green house maintained at 18 °C	Plate counting on selective medium	*E. coli* O157:H7 was not detected on the entire plant at harvest	Norway	Johannessen et al., 2005
E. coli O157:H7 ATCC 43888, non-toxigenic strain	Cabbage	Field set-up	Plate counting on rifampicin-based selective medium	Leaf surface contamination and internalisation at depended on inoculum level and time of manure application	Uganda	Ongeng et al., 2011c
E. coli O157:H7 ATCC43888, non-toxigenic strain	Cabbage	Field set-up	Plate counting on rifampicin-based selective medium	Enhanced survival in the rhizosphere was associated with long-term contamination and internalisation in cabbage leaves	Uganda	Ongeng et al., 2011d
E. coli O157:H7 B6914-GFP	Lettuce	Laboratory set-up at 20 °C	Gfp coupled with plate counting on selective medium	No contamination after 21 days of plant growth	The Netherlands	Franz et al., 2005
E. coli O157:H7 RM1484-pWM1029	Thale cress	Laboratory set-up: 16 h photoperiod, 22 °C day and 20 °C night alternation	Gfp coupled with microscopy and plate counting on selective medium	Contamination of entire plant after root inoculation, *Enterobacter asburiae* suppressed epiphytic growth of the pathogen	USA	Cooley et al., 2003
E. coli O157:H7 B6914-GFP, non-toxigenic	Carrots, onions	Field setting	Plate counting on agar medium	*E. coli* O147: H7 was detected on carrots at harvest, but not on onions	USA	Islam et al., 2005
E. coli O157:H7 B6914-GFP, non-toxigenic	Lettuce, Parsley	Field setting	Gfp coupled with plate counting on selective medium	Contamination occurred throughout the cultivation period	USA	Islam et al., 2004a
E. coli O157:H7 B6914-GFP	Lettuce	Laboratory setting at 20 °C	Gfp coupled with plate counting on selective medium	Internalisation occurred in seedlings	The Netherlands	Franz et al., 2007b
E. coli O157:H7 ATCC 43888-GFP, non-toxigenic	Spinach Parsley	Field set-up	Gfp and plate counting on selective medium	Internalisation of *E. coli* O157:H7 in the plants was infrequent	USA	Erickson et al., 2010a

Cocktail mixture of Gfp-labelled *E. coli* O157:H7 strains ATCC 43888, CV2B7, 6980-2, and 6982-2; all non-toxigenic	Spinach, lettuce	Field Set-up	Gfp coupled with plate counting on selective medium	Internalisation occurred in the leaves while survival was more on the abaxial than on adaxial side of the leaves	USA	Erickson et al., 2010b
Cocktail mixture of *E. coli* O157:H7-Gfp outbreak strains RM4407, 86-24, RM5275	Spinach	Growth chamber: 70 to 72% humidity, light intensity of 1.5 × 1-1 microeinsteins/m2/s (14 h light, 10 h dark), and 25 ± 1 °C.	Gfp coupled with plate counting on selective medium	Internalised cells observed in root tissues but not in shoot tissues during plant growth	USA	Sharma et al., 2009
A cocktail mixture of *E. coli* O157:H7-Gfp strains ATCC 43888 (human feces), EO122 (bovine isolate), K3995 (spinach isolate), K4492 (lettuce, clinical isolate), and F4546 (alfalfa sprout outbreak isolate)	Lettuce	Growth chamber set at 23 °C during the day and 7 °C at night, with a 12 h photoperiod, and light intensity of 600 to 700 mmol/m2/s.	Gfp coupled with plate counting on selective medium	No internalization of *E. coli* O157:H7 in the plant, irrespective of the type of lettuce, age of plants, or strain of *E. coli* O157:H7	USA	Zhang et al., 2009
E. coli O157:H7-Gfp strain B6-914, non-toxigenic	Spinach	The greenhouse set at 14-h photoperiod, day and night temperatures of 24 and 18 °C, and humidity of 50 and 85%	Gfp coupled with plate counting on selective medium	Surface contamination occurred depending on plant maturity but internalization was rare	USA	Pu et al., 2009
Cocktail mixture of *E.* coli O157:H7-Gfp strains E0143 (meat isolate), C7927 (human isolate), K262 (human isolate), C0083 (cattle feces isolate), and E0139 (beef jerky isolate)	Lettuce	Growth chamber for lettuce, and field set-up for lettuce	Gfp coupled with plate counting on selective medium	Internalisation and survival more on the abaxial than on the adaxial side of the leaves, exposure to insects reduced potential for internalisation	USA	Erickson et al., 2010c
E. coli O157:H7-Gfp strain ATCC 43895 (ground beef isolate)	Lettuce	Green house set at 21 °C during the day and 15 °C at night, and 12 h photoperiod	Gfp coupled with plate counting on selective medium	Leaf contamination was both superficial and internal	USA	Mootian et al., 2009

Table 5. Overview of reported studies on survival of *S. enterica* in the soil-plant ecosystem indicating the bacterial strains and vegetable used in the investigation, microbiological methodology applied experimental conditions, results obtained and the country of study.

Strains used	Vegetable used	Experimental conditions	Detection method	Results obtained	Country of study	Reference
A cocktail mixture of *S. Newport* RM1655, *S. typhimurium* SJW1103, *S. typhimurium* SJW 1368, *S. typhimurium* SJW1809	Thale cress	Laboratory set-up: 16 h photoperiod, 22 °C day and 20 °C night alternation	Gfp coupled with microscopy and plate counting on selective medium	Contamination of entire plant after root inoculation, *Enterobacter asburiae* suppressed epiphytic growth of the pathogen	USA	Cooley et al., 2003
S. typhimurium (χ^{3985} Δcrp-11 Δ cya-12), non-virulent	Carrots, radishes	Field set-up	Plate counting on selective medium	Salmonella was detected on carrots and radish throughout the cultivation period	USA	Islam et al., 2004b
S. Montevideo, bioluminescent construct	Mung bean	Laboratory set-up	Bioluminescent measurement	Superficial and endophytic colonisation of the sprouts	England	Warriner et al., 2003b
S. typhimurium LT2, laboratory attenuated	Cabbage	Field set-up	Plate counting on rifampicin-based selective medium	Leaf surface contamination and internalisation at depended on inoculum level and time of manure application	Uganda	Ongeng et al., 2011c
S. typhimurium LT2, laboratory attenuated	Cabbage	Field set-up	Plate counting on rifampicin-based selective medium	Enhanced survival in the rhizosphere was associated with long-term contamination and internalisation in cabbage leaves	Uganda	Ongeng et al., 2011d
S. typhimurium (χ^{3985} Δcrp-11 Δ cya-12), non-virulent	Lettuce, Parsley	Field set-up	Plate counting on selective medium	Vegetable contamination occurred throughout cultivation period	USA	Islam et al., 2004c
S. Weltevreden strain 2007-60-3289-1	Spinach	Phytotron:20/12 °C, light/dark cycle of 18 h/16 h, relative humidity of 70% and light intensity of 400 µmol photons $m^{-2} s^{-1}$	qPCR	*S. Weltevreden* persisted in the entire soil-plant ecosystem for at least 21 days, but was more associated with the roots	Sweden	Arthurson et al., 2011

the work of Ongeng et al. (2011c,d) suggest that the organisms are capable of withstanding the intracellular inert defence mechanisms of the plant as well as competing with other seasoned endophytes for nutrients and attachment sites. Considering that internal tissues of plants are not the primary habitat of human enteric pathogenic bacteria, future studies could look at adaptation phenomena that enable *E. coli* O157:H7 and/or *S. typhimurium* to efficiently colonize and survive endophytically in plant tissues. Finally, certain insects appear to deter internalization of enteric pathogenic bacteria in vegetable tissues. Erickson et al. (2010c) showed that brief exposure (~18 h) of lettuce leaves to insects cabbage loopers, thrips or aphids prior to inoculation with *E. coli* O157:H7 resulted in significantly reduced internalized populations of the organism within the leaves after approximately 2 weeks, as compared with leaves that were not exposed to insects. The authors never went ahead to elucidate on the mechanism behind the reduced internalization phenomenon in the presence of the insects. But nevertheless, their results suggest that those insects could have produced or caused the plant to produce certain substances that are antagonistic to *E. coli* O157:H7. This is a potential subject for future research.

The phylosphere is a very harsh ecological niche. This niche is characterized by low moisture, low nutrient availability and fluctuating temperature and osmotic conditions in addition to being exposed to solar radiation. In order for *E. coli* O157:H7 and/or *S. enterica* to survive on the leaves of vegetable crops in the field, the organisms should be able to compete with other epiphytes for the limited nutrients and moisture and to withstand the effects of solar radiation as well as the mechanical effects of rainfall. A number of studies have reported long-term occurrence of *E. coli* O157:H7 and *S. enterica* on the phylospheres of various fresh vegetable crops during cultivation on contaminated soil under field conditions in various geographical regions (Erickson et al., 2010a,b,c; Ongeng et al., 2011c,d; Islam et al., 2004a,c,d). Contamination on the leaf has been reported to be more on the abaxial than on the adaxial side of the leaf (Erickson et al., 2010a,b). This might be attributed to the higher availability of moisture and less exposure of the abaxial surface to solar radiation than is the case for the adaxial side. It is apparently not known whether the long-term occurrences of *E. coli* O157:H7 and/or *S. enterica* on the phylosphere observed in studies mentioned above were genuinely due to the capacity of the organisms to survive on the leaves or were just simply due to frequent deposition of new contaminants from the soil by rain splash or other vectors including slugs (Sproston et al., 2006) and insects such as agromyzid flies, anthomyiid flies leafhoppers and house flies (Talley et al., 2009). This is because leaf surface contamination phenomena for vegetable crops in the field have only been observed in situations where the pathogens were present in the soil, but not otherwise.

The first step in the colonization of the plant surfaces by enteric pathogenic bacteria such as *E. coli* O157:H7 and/or *S. typhimurium* involve the attachment of the organism to the site of contact. Several genetically coded factors appear to be involved in the attachment and binding of the two enteric bacteria to plant surfaces. In *E. coli* O157:H7, the EsPA fiber (Shaw et al., 2008), the Type Three Secretion System (Xicohtencatl-Cortes et al., 2009), the curli, the *E. coli* common pilus, hemorrhagic coli type 4 pilus and flagella (Saldaña et al., 2011), cellulose, poly-β-1,6-*N*-Acetyl-D-Glucosamine, and colanic acid (Matthysse et al., 2008) have been shown to enhance attachment and optimal binding of the organism to leafy vegetables. In the case of *S. enterica*, the curli and the stationary phase sigma factor, the elements important in the virulence of the organism to animal host, were found to be important in the attachment process (Barak et al., 2005). The epiphytic fitness of enteric bacteria such as *E. coli* O157:H7 and *S. enterica* on plant leaves has also been attributed to the ability of the organisms to form biofilms. This aspect has recently been reviewed by Steenackers et al. (2011) and shall not be discussed further in this review.

The binding pattern of *S. enterica* on plant surfaces appears to be serovar-dependent while the tenacity of attachment seems to be serovar-independent. Serovar dependency of the attachment pattern was observed in a study which demonstrated that serovars Typhimurium and Senftenberg bound to the surface of unripe tomatoes in an aggregative pattern while serovar Thompson adhered diffusely instead (Shaw et al., 2011). Homogeneity in attachment tenacity was observed on alfalfa sprouts using serovars Havana, Cubana Badilon, Mbandaka, Newport, Poona and Schwarzenground (Barak et al., 2002). In the same study *S. enterica* serovars were found to attach and grow on alfalfa sprouts better than *E. coli* O157:H7 strains thus suggesting differences in ecological fitness between *E. coli* O157:H7 and *S. enterica*. Future research could explore the fundamental basis for the observed differences. Nevertheless, the growth of *E. coli* O157:H7 and/or *S. enterica* on alfalfa sprouts observed in the work of Barak et al. (2002) indicate that the two species of enteric bacteria are indeed capable of salvaging nutrients in competition with diverse microbial communities present on the phylosphere. In-depth discussion on nutrient acquisition by organisms living in a nutrient-deficient environment can be found in a recent review by van Elsas et al. (2011).

Potential impact of climate change variability on pre-harvest safety of field-cultivated vegetables

Crop vulnerability to the effect of climate change variability has mostly only been looked at in terms of yield losses (Nelson et al., 2009). However, the effect of climate change on microbiological safety of fresh vegetables pre-harvest has not received much attention. Climate variables such as temperature, humidity and CO_2 concentration are known to affect growth and survival of microorganisms (Martin et al., 2003). Considering that the levels of those climate variables in the agricultural environment in general are greatly influenced by their level in the atmosphere, it becomes very apparent that climate change indeed affects the behavior of human enteric bacterial pathogens such as *E. coli* O157:H7 and *S. enterica* in the agricultural environment. The immediate consequence of the climate change phenomenon is that information on the behavior of *E. coli* O157: H7 and *S. enterica* in the agricultural environment that were generated in the past will become invalid. Consequently, manure use guidelines such as the National Organic Programme of the US (Ingham et al., 2004) and the Safe

Sludge Matrix Code of the UK (Heaton & Jones, 2008) will not be able to guarantee pre-harvest safety of vegetables anymore. In addition, models that have been developed to predict survival of pathogens in the field such as the kinetic-based model of Ongeng et al. (2011e) will likely lose the prediction power. This analysis provides a theoretical background for the likely impact of climate change on microbiological safety of vegetables in the field. Experimental analysis in future should provide better understanding.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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