Minireview

Fresh fruit and vegetables as vehicles for the transmission of human pathogens

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Summary

Much research into food-borne human pathogens has focused on transmission from foods of animal origin. However, recent investigations have identified fruits and vegetables are the source of many disease outbreaks. Now believed to be a much larger contributor to produce-associated outbreaks than previously reported, norovirus outbreaks are commonly caused by contamination of foods from hands of infected workers. Although infections with Shiga toxin-producing *E. coli* O157 have been linked to beef more often than to any other food product, severe outbreaks have been traced to consumption of contaminated radish sprouts and pre-packaged spinach. Similarly, while infections with *Salmonella* have mainly been linked to consumption of foods of animal origin, many outbreaks have been traced to contaminated fresh produce. *E. coli* O157 binds to lettuce leaves by alternative mechanisms involving the filamentous type III secretions system, flagella and the pilus curli. Association of *Salmonella* with fresh produce appears to be serovar-specific involving flagella, curli, cellulose, and O antigen capsule. A better understanding of plant, microbiological, environ-

mental, processing and food handling factors that facilitate contamination will allow development of evidence-based policies, procedures and technologies aimed at reducing the risk of contamination of fresh produce.

Introduction

Fresh fruit and vegetables are important components of a healthy and balanced diet; their consumption is encouraged in many countries by government health agencies to protect against a range of illnesses such as cancers and cardiovascular diseases. However, fruits and vegetables, and in particular leafy greens that are consumed raw, are increasingly being recognized as important vehicles for transmission of human pathogens that were traditionally associated with foods of animal origin. Despite the increased importance of fresh produce as a vehicle for human pathogens, there is currently limited knowledge about where in the supply chain contamination occurs or about the mechanism by which human pathogens colonize and survive on or in fruits and vegetables. In this manuscript we review the current knowledge and possible future developments in this increasingly important area of food safety.

Epidemiology

In recent decades public health promotion of healthier lifestyles has led to increased demand for fresh produce in many industrialized nations. In the USA for example, the fresh produce industry has responded with increased domestic production, increased importation and improvements in maintaining the quality of produce for longer (Pollack, 2001). Combined, these factors have led to a 25% increase in the annual average amount (by weight) of fresh produce consumed per person during 1997–1999 compared with 1977–1979 (Pollack, 2001). This change occurred in the earlier part of that period, as little change in U.S. produce consumption occurred between 1994 and 2005 (Blanck *et al.*, 2008). Fresh produce such as fruit and salads are often consumed raw, putting consumers at

risk of infection by contaminating organisms. The fresh produce industry in many countries has responded to this by adopting various risk management practices designed to reduce the likelihood of contamination. However, despite this, the number of reported illnesses linked to contaminated produce has increased in the USA (Sivapalasingam *et al.*, 2004). Changes in agricultural, processing and distribution practices that have enhanced both the supply and range of products (e.g. triple-washing pre-packaged leafy greens) may also have increased the risk for more widespread outbreaks.

In 2007, in the European Union *Salmonella* was found in around 0.3% of produce-related samples tested (Westrell *et al.*, 2009). Large investigations on prevalence of pathogenic bacteria in fruits and vegetable were conducted in the UK, Ireland, Germany and the Netherlands in 2007 (Westrell *et al.*, 2009). The proportion of produce samples that yielded *Salmonella* in these studies ranged from 0.1% to 2.3%, with pre-cut products having some of the highest proportions contaminated. In addition, the percentage of sprouted seed samples yielding *Salmonella* was 1.5% in the Netherlands and 2.2% in Germany while 0.4–0.5% of herb and spice samples from Hungary, Netherlands and UK yielded *Salmonella.* In the USA, the proportion of all food-borne outbreaks associated with raw produce increased from 0.7% in the 1970s to 6% in the 1990s (Sivapalasingam *et al.*, 2004). Similarly, the proportion of food-borne outbreak-associated illnesses associated with raw produce increased from 1% in the 1970s to 12% in the 1990s (Sivapalasingam *et al.*, 2004). Multiple factors have contributed to these increases, including an improvement in detection. In the USA, new standard subtyping techniques for *Salmonella* and *Escherichia coli* O157 have been disseminated to all 50 states in the last decade. Public health laboratories submit molecular subtype patterns electronically to PulseNet, the national molecular surveillance network for enteric infections. This system has markedly improved the ability to detect outbreaks, especially those with widely scattered cases, and to identify their source (Gerner-Smidt *et al.*, 2006). However, it is also possible that the reported increase in the number of illnesses associated with consumption of fresh produce reflects a true increase in contamination.

The challenge for investigators is first to identify a produce item as the cause of an outbreak and then determine the mechanism of contamination. Patients often have poor recall about consumption of specific produce items and may be unable to make distinctions between various produce items. Produce is also often consumed as a mixed food product (e.g. fruit salad or mixed leaf salad), making identification of an individual item as the source of infection difficult. Even when an epidemiologic investigation can identify a produce item as a vehicle for infection, tracing the source can be difficult, due to limited labelling, mixing of product from different farms and distributors, incomplete or discarded records, and increased globalization of produce distribution. In 1995, outbreaks of *Salmonella* serovar Stanley infections caused by alfalfa sprouts were identified simultaneously in the USA and Finland. The source of inoculum for both was thought to be contaminated seeds from a Dutch shipper which could have received contaminated seeds from either of two continents (Mahon *et al.*, 1997).

A wide spectrum of pathogens and vehicles for their dissemination has been documented in produce-associated outbreaks. A review of produce-associated outbreaks in the USA from 1973–1997 found that viruses accounted for only 20% of outbreaks in which an agent was identified, most due to hepatitis A (Sivapalasingam *et al.*, 2004). Since then, improvements in diagnostics have led to a surge in reported norovirus outbreaks. Norovirus is now widely viewed as the leading cause of food-borne illness and is likely to be a much larger contributor to produce-associated outbreaks than previously reported (Widdowson *et al.*, 2005). Most reported norovirus and hepatitis A outbreaks have been due to contamination of foods from the hands of infected workers at or close to the point of service, but other modes of transmission occur. Multiple outbreaks of norovirus gastroenteritis associated with raspberries have been reported, especially in Europe (Cotterelle *et al.*, 2005; Falkenhorst *et al.*, 2005; Korsager *et al.*, 2005; Hjertqvist *et al.*, 2006). Similarly, outbreaks of hepatitis A infection have been reported as associated with raspberries, strawberries, green onions and lettuce (Reid and Robinson, 1987; Rosenblum *et al.*, 1990; Hutin *et al.*, 1999; Dentinger *et al.*, 2001). Proposed mechanisms of contamination in these outbreaks have included use of contaminated water and handling of produce by infected individuals at the farm or post harvesting level.

Parasites accounted for 16% of all produce-associated outbreaks reported in the USA from 1973–1997 (Sivapalasingam *et al.*, 2004). Among them, *Cyclospora was* the parasite most commonly reported. Multiple outbreaks of cyclosporiasis associated with raspberries have been documented particularly during the 1990s (Herwaldt and Ackers, 1997; Koumans *et al.*, 1998; Herwaldt and Beach, 1999; Herwaldt, 2000; Ho *et al.*, 2002). Most of the US outbreaks were linked to raspberries imported from Guatemala.

Bacterial pathogens continue to be a major contributor to produce-associated food-borne illnesses. In a review of produce-associated outbreaks in the USA from 1973–1997, bacteria were responsible for 60% of outbreaks in which an etiologic agent was identified (Sivapalasingam *et al.*, 2004). *Salmonella* was the most commonly reported bacterial pathogen, accounting for nearly half of the outbreaks due to bacteria (Sivapalasingam *et al.*, 2004).

A wide spectrum of produce vehicles have been associated with *Salmonella* infections. Several large-scale outbreaks have been linked to consumption of tomatoes (Hedberg *et al.*, 1999; Cummings *et al.*, 2001; Gupta *et al.*, 2007; Greene *et al.*, 2008) and melons (Gayler *et al.*, 1955; Mohle-Boetani *et al.*, 1999; Bowen *et al.*, 2006; Munnoch *et al.*, 2009). In 2008, jalapeño and serrano peppers were vehicles for a large multistate outbreak of *Salmonella* serovar Saintpaul infections. (Centers for Disease Control and Prevention, 2008). Examples of other outbreaks of *Salmonella enterica* linked to ready-to-eat plant produce include an outbreak in Scandinavia and the UK of serovar Thompson infections associated with consumption of rocket leaves (Nygard *et al.*, 2008); an outbreak of serovar Anatum infections in Denmark linked to imported basil (Pakalniskiene *et al.*, 2009); and an outbreak of serovar Senftenberg infection associated with imported Israeli basil affecting the UK, Denmark, the Netherlands and the USA (Pezzoli *et al.*, 2008).

Multiple outbreaks of *Salmonella* and *E. coli* O157 infections have been associated with sprouted seeds (Mahon *et al.*, 1997; Michino *et al.*, 1999; Taormina *et al.*, 1999; Van Beneden *et al.*, 1999; Breuer *et al.*, 2001; Honish and Nguyen, 2001; Mohle-Boetani *et al.*, 2001; 2009; Proctor *et al.*, 2001; Stratton *et al.*, 2001; Centers for Disease Control and Prevention, 2002; van Duynhoven *et al.*, 2002; Winthrop *et al.*, 2003; Ferguson *et al.*, 2005; Emberland *et al.*, 2007; Erickson and Doyle, 2007; Werner *et al.*, 2007). Most of these outbreaks have been attributed to contaminated seeds, which are often distributed across a wide geographic area. Unpasteurized juice including cider has been responsible for multiple outbreaks of *Salmonella* and *E. coli* O157 infections (Besser *et al.*, 1993; Cook *et al.*, 1998; Krause *et al.*, 2001; Vojdani *et al.*, 2008; Jain *et al.*, 2009).

Produce-associated outbreaks of *E. coli* O157 infections linked to consumption of leafy green vegetables have been increasingly recognized (Ackers *et al.*, 1998; Hilborn *et al.*, 1999; Friesema *et al.*, 2008; Grant *et al.*, 2008; Soderstrom *et al.*, 2008; Wendel *et al.*, 2009). In particular, in September 2006, an outbreak associated with consumption of pre-packaged spinach occurred across 26 states in the USA, resulting in 183 confirmed infections and three deaths (Centers for Disease Control and Prevention, 2006; Grant *et al.*, 2008; Wendel *et al.*, 2009). The largest *E. coli* O157 outbreak to date, in 1996 centred in Sakai City, Osaka, Japan, was traced to consumption of white radish sprouts (Michino *et al.*, 1999).

Sources of plant contamination

Numerous studies have investigated the potential sources of produce contamination in the supply chain both at the pre-harvest (in the field) and post-harvest stages. During the pre-harvest phase, pathogen populations can establish themselves on growing crops. The risk can be amplified after harvest either by further direct contamination or by proliferation of existing pathogen populations during processing and post harvest handling procedures.

Water is likely to be an important source of contamination in the field. Possible sources are run-off from nearby animal pastures and irrigation from a contaminated source. The risk associated with using water from a range of sources that vary in microbiological quality for irrigation of produce has been assessed and the need for improved guidelines recognized (Hamilton *et al.*, 2006; Tyrrel *et al.*, 2006). While studies have found no internalization of *E. coli* O157:H7 in spinach plants through contaminated soil, uptake and internalization was found in spinach leaves after contaminated water was dropped on the leaves (Mitra *et al.*, 2009). This suggests a lower likelihood of transmitting pathogen from contaminated water through drip irrigation versus a higher likelihood through overhead sprinkler systems. However, irrigation is not the only reported route of contamination linked to water. Use of water in postharvest processing has also played a role. An outbreak of infections with *Salmonella* serovar Newport was linked to consumption of mangoes treated with a process involving hot water aimed at preventing importation of fruit flies (Sivapalasingam *et al.*, 2003).

Pathogens may be transferred to the environment by application of inadequately composted or raw animal manures or sewage (Beuchat and Ryu, 1997; Roever, 1998; Natvig *et al.*, 2002; Santamaria and Toranzos, 2003). The faeces of wild animals may also be a source (Rice *et al.*, 1995; Ackers *et al.*, 1998). In the US outbreak of *E. coli* O157 infections associated with pre-packaged spinach, trace-back and environmental investigations determined that one ranch in California's Salinas Valley was the likely source of the outbreak. The patterns produced by pulsed-field gel electrophoresis (PFGE) and multilocus variable number tandem repeat analysis (MLVA) from the strains involved in the outbreak matched those from isolates recovered from local feral swine and cattle faeces (Jay *et al.*, 2007). However, the manner in which the spinach became contaminated was not determined.

Insects are also a possible source of contamination. In laboratory conditions, contaminated flies have been shown to directly transfer bacteria to plant leaves or fruits (Iwasa *et al.*, 1999; Sela *et al.*, 2005; Talley *et al.*, 2009). Studies have implicated flies in contamination of leaves by *E. coli* O157:H7 (Iwasa *et al.*, 1999). Large numbers of flies belonging to the *Muscidae* and *Calliphoridae* families which were found in production fields adjacent to rangeland habitats occupied by cattle were shown to carry *E. coli* O157:H7 (Talley *et al.*, 2009).

Proposed sources of contamination of fruit used for juice have included the use of fallen fruit that has been in

contact with contaminated soil, water, sewage or manure, use of contaminated water in washing or processing fruit, and contamination at the point of consumption (Vojdani *et al.*, 2008).

Post harvesting processes, ranging from storage and rinsing to cutting, are also possible sources of contamination (Wachtel and Charkowski, 2002). Cut surfaces of leaves are a specific target for pathogenic bacteria such as *Salmonella*, which show a specific tropism towards them (Kroupitski *et al.*, 2009a), and cutting melons may carry pathogens from the rind onto the edible part of the fruit where bacteria may multiply if the cut melon is not refrigerated (Ukuku and Sapers, 2007). The use of inadequately decontaminated water in hydrocoolers, which are used to store and process large quantities of fresh produce, can lead to contamination of an entire lot (Gagliardi *et al.*, 2003).

Mechanism of microbial adhesion

Attachment is a pre requisite for colonization and subsequent transmission of pathogens via the edible parts of plants. Indeed, once attached it is very difficult to remove the pathogens from contaminated fruit and vegetables by washing (Beuchat and Scouten, 2002). In this section we review the mechanisms that bacterial pathogens use to colonize fruit, vegetables and salad leaves.

Salmonella

This genus is composed of two species; *S. enterica* and *S. bongori* (reviewed in Su and Chiu, 2007). *Salmonella enterica*, which is a leading cause of gastroenteritis, is subdivided into hundreds of serovars (reviewed in Lan *et al.*, 2009). It is the pathogen most frequently linked to consumption of fruit and vegetables (Sivapalasingam *et al.*, 2004). *S. enterica* serovars can colonize seeds (Mahon *et al.*, 1997; Winthrop *et al.*, 2003), sprouted seeds (O'Mahony *et al.*, 1990), leaves (Campbell *et al.*, 2001; Horby *et al.*, 2003) and fruit (Mohle-Boetani *et al.*, 1999; Guo *et al.*, 2001) of a variety of plant species.

A survey of a range of *S. enterica* serovars revealed that while Typhimurium, Enteritidis and Senftenberg adhered efficiently to leafy vegetables, others (Arizona, Heidelberg and Agona) did not (Berger *et al.*, 2009a). Moreover, the mechanisms of adhesion among the different serovars appear to differ. Barak *et al.* reported that the pilus curli (also known as tafi) (encoded by *agfB*) play an important role in adhesion of serovars Enteridis and Newport to alfalfa sprouts. However, they also found that deletion of *agfB* did not prevent leaf attachment completely, indicating that other adhesins likely play a role (Barak *et al.*, 2005). Further studies have shown that the O antigen capsule (encoded by *yihO*) and cellulose syn-

thesis (encoded by *bcsA*) also play a role in adhesion of serovar Enteritidis (Barak *et al.*, 2007). Curli and cellulose can also play a role during transfer of serovar Typhimurium from contaminated water to parsley (Lapidot and Yaron, 2009). Interestingly, curli, cellulose and capsule are regulated by *AgfD* which may play a major role in environmental fitness of *Salmonella* (Gibson *et al.*, 2006). Curli and cellulose have been consistently found to form a cellular matrix, which allows formation of biofilms (Jonas *et al.*, 2007). *Salmonella* strains that form extensive biofilm were found to have stronger adhesion to Romaine lettuce leaves and greater persistence on intact lettuce leaves at 4°C over 9 days than weak biofilm-producing strains (Kroupitski *et al.*, 2009a).

In 2007 an outbreak of *Salmonella* serovar Senftenberg infections in the UK, Denmark, the Netherlands and the USA was traced to consumption of basil imported from Israel (Pezzoli *et al.*, 2008). Studies using the outbreak strain suggested that the bacterial flagellae play a major role in adherence of serovar Senftenberg to leaves but not serovar Typhimurium (Berger *et al.*, 2009a). However, a recent elegant study (Kroupitski *et al.*, 2009b) has shown that flagellae play a role in invasion of serovar Typhimurium into the mesophyll; inoculation of iceberg lettuce leaves in the light, but not in the dark, resulted in bacterial aggregation around stomata and invasion into the inner leaf tissue. *Salmonella* serovar Montenegro internalized into bean sprout seed has been detected inside the growing plant after germination (Warriner *et al.*, 2003); this suggests that *Salmonella* strains can 'invade' plant tissues as well as adhere to plant surfaces.

Escherichia coli

Escherichia coli is the most predominant bacteria of the aerobic gut normal flora (reviewed in Farthing, 2004). However, several *E. coli* clones have acquired pathogenicity islands via horizontal gene transfer that enable them to cause urinary tract infections and diarrheal diseases. Diarrheal *E. coli* are divided into six categories that can cause illness ranging from moderate diarrhea to severe systemic diseases (e.g. haemolytic uremic syndrome) (Palermo *et al.*, 2009).

Shiga toxin-producing E. coli

Shiga toxin-producing *E. coli* (STEC) is a zoonotic pathogen colonizing mainly cattle and small ruminants. Although cattle products, principally beef, are the most commonly recognized sources of *E. coli* O157 infections, fruits and vegetables consumed raw are also an important source (Rangel *et al.*, 2005).

Three leaf attachment mechanisms have been described in *E. coli* O157. First, contrary to non-pathogenic *E. coli*, STEC O157:H7 adhere strongly to
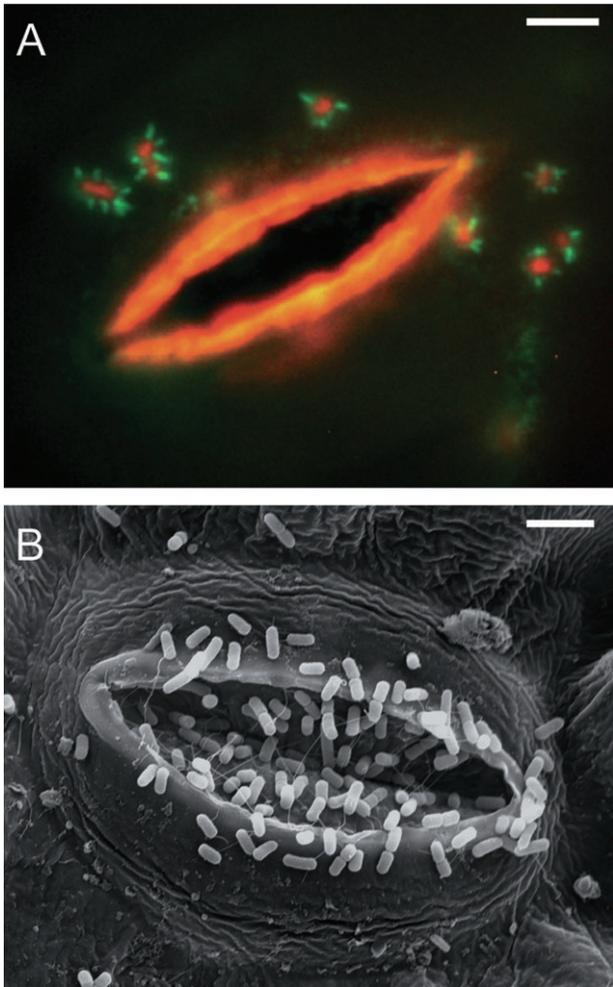
Fig. 1. Adhesion of enteric pathogens to fresh salad leaves.
A. Immunofluorescence staining, using primary anti-EspA antibodies and secondary anti-rabbit antibodies, showing EspA filaments (green) linking propidium iodide stained EPEC (strain E2348/69) (red) with the leaf epidermis (not shown). Propidium iodide counterstaining of the stomatal guard cells is shown in red.
B. Scanning electron micrograph showing binding of EAEC (strain 1917) to guard cells of the leaf stomata and bacteria reaching the sub-stomatal cavity. Bars = 0.5 μm.

tomato skin, spinach leaves and roots of alfalfa sprouts. Adhesion to these surfaces is mediated by curli (Jeter and Matthysse, 2005). Expression of curli on the surface of non-pathogenic *E. coli* was shown to be sufficient to enable bacterial attachment to alfalfa roots, but deletion of curli genes in *E. coli* O157 did not abolish adhesion, suggesting that other attachment factors were involved (Jeter and Matthysse, 2005). Second, Shaw and colleagues (2008) have shown that adhesion of *E. coli* O157, as well as the related enteropathogenic *E. coli* (EPEC), to a variety of salad leaves is mediated by the filamentous type III secretion system (T3SS), which is composed of EspA filaments (Knutton, 1995) (Fig. 1A). The *E. coli* O157 T3SS is designed to translocate effector proteins

into mammalian cells; protein translocation is mediated by an ATPase (EscN) and dependent on a translocation pore that is inserted into the plasma membrane (formed by EspB and EspD (reviewed in Garmendia *et al.*, 2005). While deletion of *escN* or *espA* resulted in loss of leaf attachment activity, adhesion of *E. coli* O157 to salad leaves was independent of protein translocation as deletion of *espB* did not affect the level of adhesion (Shaw *et al.*, 2008; Xicohtencatl-Cortes *et al.*, 2009). Importantly, while wild-type *E. coli* O157 adhered diffusely to the epidermis and aggregated around the stomata, deletion of *espB*, while not affecting the global level of adhesion, resulted in a loss of stomatal tropism. Finally, Xicohtencatl-Cortes *et al.* have shown that flagella also play a role in *E. coli* O157 leaf attachment as deletion of *fliC* encoding flagellin reduced the level of adhesion (Xicohtencatl-Cortes *et al.*, 2009). Taken together these data suggest that *E. coli* O157 employ multiple mechanisms to colonize plants and are well adapted to this biosphere. Like *Salmonella*, *E. coli* O157 can also reach the sub-stomatal cavity and the spongy mesophyll and survive in this environment (Itoh *et al.*, 1998; Solomon *et al.*, 2002a; Wachtel *et al.*, 2002; Warriner *et al.*, 2003; Jablasone *et al.*, 2005; Franz *et al.*, 2007; Xicohtencatl-Cortes *et al.*, 2009).

Enteroaggregative E. coli *and enterotoxigenic* E. coli

Enteroaggregative *E. coli* (EAEC) may be an important cause of bacterial gastroenteritis in the USA (Wilson *et al.*, 2001; Nataro *et al.*, 2006). However, the reservoir of EAEC is unknown. Recently, Berger and colleagues (2009b) observed the following two patterns of bacterial distribution following 1 h incubation of EAEC with lettuce leaves: (i) diffuse adherence to the epidermis and (ii) localized adhesion to the guard cell of the stomata (Fig. 1B). Studies employing specific mutants revealed that binding to the epidermis is mediated by the AAF pilus, which is known to play a role in colonization of the human gut (Berger *et al.* 2009b) while aggregation around the stomata is mediated by flagellae.

Enterotoxigenic *E. coli* (ETEC) is an important cause of infantile and travelers' diarrhea (Nataro and Kaper, 1998; Qadri *et al.*, 2005) and causes severe watery diarrhea in calves and piglets (Nagy and Fekete, 2005). One study reported that the flagella is the main adhesin mediating attachment of ETEC to the epidermis of lettuce leaves (Shaw *et al.*, 2010).

Survival

The phyllosphere (the total above-ground surfaces of plants) is characterized by a number of extreme and often fluctuating environmental conditions combined with

unique physio-chemical characteristics, to which typical phyllosphere microorganisms develop adaptations that allow them to grow in these habitats. Human pathogens are not normally considered to be a part of the phyllosphere microbial population but it is clear that they can be, as evidenced by the outbreaks of food-borne illness described above.

Many studies have been conducted on the behaviour and survival of human pathogens on plants. Most have focused on *E. coli* (largely *E. coli* O157:H7) and *Salmonella* sprayed or applied directly onto the foliage of plants by a range of techniques or applied on seeds, roots or into soil. With so many different experimental systems it is difficult to make general statements concerning population dynamics and survival of human pathogens on crop plants. Nevertheless, when applied directly to foliage, both *E. coli* and *Salmonella* can survive on parsley in the field for 177 and 231 days respectively (Islam *et al.*, 2004a,b). Spraying lettuce plants with water contaminated with *E. coli* O157:H7 resulted in recovery of the pathogen on foliage 30 days later (Solomon *et al.*, 2003). Surface irrigation and spray irrigation with suspensions of *E. coli* O157:H7 also led to recovery of the pathogen from lettuce tissue; the level of contamination was lower from drip than from sprinkler irrigation. The lettuce leaves remained contaminated with *E. coli* O157:H7 even after washing, indicating that surface and spray irrigation of food crops with water of unknown microbiological quality poses important hazards (Solomon *et al.*, 2002b).

In a single study, six human pathogens including bacteria and viruses were all found to survive for 14 days on the phyllosphere of cantaloupe, lettuce and pepper under controlled environment conditions (Stine *et al.*, 2005), demonstrating that human pathogens can survive in this ecological niche on important crop plants for commercially relevant periods.

The phyllosphere is not the only ecological niche in which human pathogens are associated with plants. Whereas no evidence of uptake of *E. coli* O157 from soil to internal plant tissue was observed (Jablasone *et al.*, 2005; Sharma *et al.*, 2009), reports suggest that plant roots could be colonized by *E. coli* O157 and *Salmonella* (Wachtel *et al.*, 2002; Islam *et al.*, 2004a,b; Jablasone *et al.*, 2005; Jeter and Matthysse, 2005).

Storage temperature affects the survival and growth of pathogens on cut surfaces of produce items. After a large outbreak of *Shigella* associated with shredded lettuce was identified, laboratory studies demonstrated the rapid growth of *Shigella* on shredded lettuce stored at temperatures that were too warm (Davis *et al.*, 1988). In another study, various formulations of salsas were found to support rapid growth *Salmonella* when stored at warm temperatures, particularly if jalapeño peppers were included;

growth was slower if fresh garlic and lime juice were part of the formulation (Ma *et al.*, 2010).

Plant defence

When enteric pathogens colonize plant surfaces or sub-stomatal cavities, they need to avoid or negate the plant's defence mechanisms. Plants have a large range of sensors which allow them to detect and initiate measures to control undesired bacterial proliferation. *Arabidopsis thaliana* has been used extensively as a model plant for studies to understand these defence mechanisms due to the available genome sequence and the relative ease with which it can be genetically manipulated.

During the last 10 years, a series of pathogen-associated molecular pattern (PAMP) receptors have been identified for flagelin and EF-Tu (reviewed in Nurnberger and Kemmerling, 2006). Interestingly, leaves infected or infiltrated with *E. coli* O157:H7 showed no obvious effects and the bacteria neither multiplied nor died (Thilmony *et al.*, 2006). Global genetic analysis revealed that leaf infiltration leads to up regulation of bacterial genes, although to a lower extent than with the plant pathogen *Pseudomonas syringae*. One of the better-characterized PAMP receptors is FLS2, which recognizes a 22 amino acid region in the amino terminus of the flagella (Asai *et al.*, 2002). Although 18 of the 22 amino acid residues are identical between *E. coli* O157 and *P. syringae*, the *E. coli* flagellae do not induce any specific plant response (Thilmony *et al.*, 2006). In addition, the plant hormone ethylene plays a role in defences against plant pathogens (review in Broekaert *et al.*, 2006); while *P. syringae* induced a high level of ethylene expression, *E. coli* O157 induced a 10-fold lower transcription (Thilmony *et al.*, 2006), suggesting that either the plant does not sense the presence of the bacteria or that the bacteria inhibit activation.

In a similar manner, although *Salmonella* serovar Typhimurium colonizes the roots and leaves of various plants and induces a host response (Iniguez *et al.*, 2005; Schikora *et al.*, 2008), this response is much lower than that induced by *P. syringae*. Deletion of *Salmonella* serovar Typhimurium genes encoding flagellar and Spi1 T3SS components decreased plant response and in parallel increased the colonization (Iniguez *et al.*, 2005). These results suggest that although plants may be able to modulate colonization by human enteric pathogens, they do not recognize them as potentially harmful and so do not initiate defence mechanisms to prevent colonization. Human enteric pathogens therefore appear to be able to exist as a component of the phyllosphere microbial community both as epiphytes on leaf surfaces and endophytes within leaf tissues.

Plant genetics

While environmental and bacterial genetic factors play a critical role in determining patterns of phyllosphere colonization by bacteria, including human pathogens, much less is known of plant-related factors that determine the potential for human pathogens to colonize, grow or survive as epiphytes or endophytes on or in leaves. Development of the microbial population in the phyllosphere is influenced by phenotypic characteristics of the host plant and it is possible to observe 'hot-spots' of microbial growth at specific sites on the leaf (Kinkel, 1997). Gross plant morphology is known to influence the size of phyllosphere bacterial populations (Thompson *et al.*, 1993) and variation in bacterial populations between different species have been attributed to a range of plant characteristics, including leaf water content, leaf P content, amounts of bacteria-inhibitory phenolics, and leaf and mesophyll thickness (Yadav *et al.*, 2005). There have been very few studies of plant cultivar effects on human pathogens; however a recent report demonstrated a differential interaction between lettuce cultivars and *S. enterica* serovars (Klerks *et al.*, 2007).

Prevention

Food safety has major implications on human health, social behaviour and economy. Food-borne disease outbreaks can make substantial numbers of people ill while attendant recalls and publicity can reduce consumer confidence and decrease demand with significant economic loss for all parts of the supply chain. For these reasons, many producers and suppliers are committed to good practices to reduce the risk of contamination. Conventional methods to decrease contamination include post harvest decontamination procedures. However, experiments using standard post-harvest decontamination procedures with solutions containing approximately 20–200 μg ml^{-1} free chlorine for various lengths of time found that bacterial numbers were reduced but the treatments did not completely eliminate either the natural microbial population or human pathogens (Seo and Frank, 1999; Lang *et al.*, 2004).

Household natural sanitizers including fresh lemon juice and vinegar have been shown to have some effect in the reduction of *Salmonella* serovar Typhimurium on rocket leaves and spring onion. A 15 min treatment with 1:1 lemon juice and vinegar reduced viable counts to undetectable levels (Sengun and Karapinar, 2005). Treatment of carrots with this solution reduced *Salmonella* CFUs to an undetectable level (Sengun and Karapinar, 2004). Treatment of commercial iceberg lettuce preinoculated with natural spoilage organisms with chlorine, ozone or their combination reduce the number of viable micro-

organism (Garcia *et al.*, 2003). Additionally chlorine-ozone combinations increased the shelf life of lettuce. No visible changes in rinse water turbidity or reduction in quality were observed during rinsing of the lettuce, indicating applicability to commercial processing (Garcia *et al.*, 2006).

Ionizing radiation has demonstrated efficacy in reducing microbial contamination. In particular, studies focused on leafy greens have shown multiple log reductions in *Listeria monocytogenes*, *Salmonella* and *E. coli* O157:H7 when used on various leafy greens including iceberg lettuce, Romaine lettuce and spinach (Niemira *et al.*, 2003; Niemira, 2007; 2008). An alternative method to reduce contamination would be the use of agricultural practices that encourage growth of competing bacteria within the phyllosphere to reduce the contamination with human pathogens. Various studies suggest that the natural microflora of plants can inhibit growth of *E. coli* O157:H7, *Salmonella serovars* Montevideo and Chester, and *Staphylococcus aureus* (Schuenzel and Harrison, 2002; Johnston *et al.*, 2009). *Pseudomonas* and *Bacilus* species isolated from green pepper, Romaine lettuce, baby carrots, alfafa and clover sprouts can inhibit growth of *Salmonella* serovar Chester and *L. monocytogenes* (Liao and Fett, 2001). *Enterobacter cloacae* reduced colonization of carrots, cress, lettuce, radish, spinach, and tomatoes by *E. coli* O157:H7 and *L. monocytogenes* (Jablasone *et al.*, 2005), whereas *Enterobacter asburiae* decreased their survival on lettuce (Cooley *et al.*, 2006). Also, growth of *Arabidopsis thaliana* with *E. asburiae* in gnotobiotic conditions strongly reduced root contamination by *Salmonella* or *E. coli* O157 (Cooley *et al.*, 2003).

Conclusion

Ensuring the security of current and future food supplies is one of the main challenges facing governments around the world, driven by the need to feed an increasing world population and consumer demand for freshness and variety. However, there is also a need to address issues associated with the supply of safe and healthy food. In particular, pressure on agricultural land due to urban expansion, predicted global warming, increased frequency of flooding in some regions and drought in others, and the need to reduce the carbon footprint associated with food production is likely to drive important changes in land use and agricultural production practices, some of which (e.g. bringing animal and crop production closer together and the need to recycle water for irrigation) may have an impact on food safety.

Governments around the world have been promoting consumption of fresh vegetables and fruits as part of a healthy diet to prevent disease. In parallel, the fresh produce retail industry has developed value added

products such as bagged salad, which meet consumer demands for convenience and variety. Paralleling this has been an increased recognition of food-borne outbreaks linked to the consumption of fresh and ready-to-eat vegetables, herbs and fruits.

Prevention efforts should continue to focus on good agricultural practices, improved traceability and good manufacturing practices. Concepts similar to hazard analysis and critical control point programs, which have been successful in other areas of food production, if systematically applied in crop production industries, could increase food safety. A better understanding of plant, microbiological, environmental, farm, processing and food handling factors that interact with one another to determine whether contamination occurs, and whether pathogens survive or proliferate will support the development of evidence-based policies, procedures, and technologies aimed at improving the safety of fresh produce. This includes knowledge about attachment, colonization and internalization by human pathogens of leaf tissues. Further research is required to provide the underpinning knowledge needed for an evidence-based approach to replace the current empirical approach.

Acknowledgements

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