CHAPTER 4

Recent Advances in the Microbial Safety of Fresh Fruits and Vegetables

Keith Warriner,* **Ann Huber,†** **Azadeh Namvar,*** **Wei Fan,*** and **Kari Dunfield‡**

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* Department of Food Science, University of Guelph, Guelph, Ontario, Canada
† Soil Resource Group, Guelph, Ontario, Canada
‡ Department of Land Resources, University of Guelph, Guelph, Ontario, Canada

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Abstract Foodborne illness outbreaks linked to fresh produce are becoming more frequent and widespread. High impact outbreaks, such as that associated with spinach contaminated with *Escherichia coli* O157:H7, resulted in almost 200 cases of foodborne illness across North America and >$300 m market losses. Over the last decade there has been intensive research into gaining an understanding on the interactions of human pathogens with plants and how microbiological safety of fresh produce can be improved. The following review will provide an update on the food safety issues linked to fresh produce. An overview of recent foodborne illness outbreaks linked to fresh produce. The types of human pathogens encountered will be described and how they can be transferred from their normal animal or human host to fresh produce. The interaction of human pathogens with growing plants will be discussed, in addition to novel intervention methods to enhance the microbiological safety of fresh produce.

I. INTRODUCTION

The fresh-cut market has experienced rapid growth within the last decade and an estimated 6 million packs of bagged produce are sold within North America each day (Doyle and Erickson, 2008; Jongen, 2005). The driving force behind the rapid growth of the fresh produce is the desire of consumers to lead a healthy lifestyle along with the convenience of pre-prepared products. Consumers have become accustom to all-year-around availability of fruit and vegetables with the convenience of prepacked products that require minimal preparation. To meet consumer demand, the primary production has shifted away from local farmers to highly centralized centers, which in the case of North America, are located in Mexico, California, and Florida (Doyle and Erickson, 2008). Indeed, California alone supplies over 70% of all the leafy greens (e.g., lettuce, spinach) consumed within North America (FDA, 2002). Centralization of production has brought many benefits to the consumer such as relatively cheap produce, consistent quality, and all-year-round availability. However, the downside of centralized production is that when foodborne illness outbreaks occur, they are typically widespread and involve a high number of

cases (Gorny, 2006). Although consumers in the course of an outbreak frequently turn to organic or locally grown produce, there is no evidence that this poses any less risk compared to 'conventionally' produced crops (Arthur *et al.*, 2007; Loncarevic *et al.*, 2005). In this respect, it cannot be concluded that the rapid rise in foodborne illness outbreaks are linked to centralized production alone. It is noteworthy that the increase in the fresh-cut (bagged salad) market has coincided with the increase in foodborne illness cases. Here, the produce is cut or shredded which provides entry points for pathogens, in addition to releasing nutrients to support microbial growth. Modified Atmospheric Packaging (MAP) reduces spoilage by aerobes but can enhance the virulence of pathogens such as *Escherichia coli* O157:H7 (Chua *et al.*, 2008). In addition, the higher proportion of vulnerable, susceptible, people within a population along with increased surveillance and high prevalence of virulent pathogens within the environment are further reasons to consider for the rise in foodborne illness outbreaks (Arthur *et al.*, 2008; FDA, 2001; Sewell and Farber, 2001).

II. OUTBREAKS LINKED TO FRESH PRODUCE

There has been a rapid rise in foodborne illness outbreaks linked to fresh produce (Fig. 4.1). The pathogens of main concern are *Salmonella* and *E. coli* O157:H7 although, in principle, a diverse range of pathogenic microbes can contaminate fresh produce at any point in the chain.

There has been several high profile foodborne illness outbreaks associated with fresh produce with sprouted seeds, tomatoes, and leafy greens remaining the most prominent (Table 4.1) (Doyle and Erickson, 2008). The underlying reasons for why specific produce types have been implicated in the majority of outbreaks can, in part, be explained by the market volume

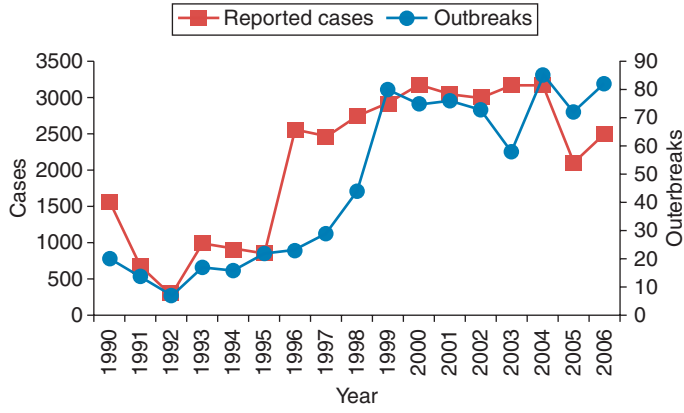

FIGURE 4.1 Foodborne illness outbreaks linked to fresh produce from 1990 to 2006. Source: Centre for Science in the Public Interest (2008).

TABLE 4.1 Outbreaks linked to fresh produce

Date	Pathogen	Produce	Comments
December 2005	*Salmonella*	Mung bean sprouts	Canada, 618 confirmed cases
February 2006	*Salmonella*	Alfalfa sprouts	Canada, sprout recall due to suspected contamination
February 2006	*Salmonella*	Alfalfa sprouts	Australia, 100 confirmed cases of salmonellosis
June 2006	*E. coli* O121:H19	Lettuce	United States, 4 confirmed cases
July 2006	*Salmonella*	Fruit salad	United States and Canada, 41 confirmed cases
August 2006	*Salmonella*	Alfalfa sprouts	United States, sprout recall due to suspected contamination
September 2006	*E. coli* O157:H7	Spinach	United States, 205 confirmed cases; 3 deaths
September 2006	*Clostridium botulinum*	Pasteurized carrot juice	United States and Canada; 6 cases
October 2006	*E. coli* O157:H7	Lettuce	Canada; 30 confirmed cases
October 2006	*E. coli* O157:H7	Lettuce	Canada; recall due to suspected contamination
October 2006	*Salmonella*	Tomatoes	United States; 183 cases
November 2006	*E. coli* O157:H7	Lettuce	United States; 81 confirmed cases
November 2006	*E. coli* O157:H7	Lettuce	United States; 71 confirmed cases
November 2006	*Salmonella*	Peanut butter	United States; 481 confirmed cases
April 2007	*Salmonella*	Lettuce	UK, recall for suspected contamination
August 2007	*Shigella sonnei*	Baby carrots	Canada, 4 cases
April 2008	*Salmonella*	Cantaloupe	Canada, United States and Mexico, 64 confirmed cases
June 2008	*Salmonella*	Tomatoes/peppers	United States and Canada, 1442 confirmed cases
September 2008	*E. coli* O157:H7	Lettuce	United States and Canada; 134 confirmed cases
September 2008	*Salmonella*	Alfalfa sprouts	United States, 14 confirmed cases
November 2008	*Salmonella*	Basil	UK, 32 confirmed cases
December 2008	*Salmonella*	Alfalfa sprouts	United States, recall for suspected contamination

(Anonymous, 2006; Thunberg *et al.*, 2002; Valentin-Bon *et al.*, 2008). However, there is a growing body of evidence supporting the hypothesis that certain pathogens are adapted to persist on different produce types (Bassett and McClure, 2008). For example, foodborne illness outbreaks linked to tomatoes have commonly implicated *Salmonella* (Barak and Liang, 2008; Greene *et al.*, 2008) (Table 4.1). The same pathogen is also associated with cantaloupes, sprouted seeds, and lettuce (Arthur *et al.*, 2007; Mohle-Boetani *et al.*, 2008; Sivapalasingam *et al.*, 2004). *E. coli* O157: H7 has been associated with sprouted seeds, lettuce, apples (juice), and spinach (Jablasone *et al.*, 2005; Sivapalasingam *et al.*, 2004; Valentin-Bon *et al.*, 2008). Parsley is prone to contamination from *Shigella* and soft fruit with enteric viruses such as Hepatitis A (Anonymous, 1999; Jacobson *et al.*, 2004; Mataragas *et al.*, 2008; Naimi *et al.*, 2003; Peterson *et al.*, 1983; Reller *et al.*, 2006; Seymour and Appleton, 2001; Wu *et al.*, 2000). A more curious associated is the link between basil and *Cyclospora* (Chacin-Bonilla, 2007; Sivapalasingam *et al.*, 2004). The underlying reasons for such associations remain obscure as is many aspects on the interactions of human pathogens with fresh produce.

Although foodborne illness outbreaks linked to fresh produce have been recorded over 30 years, there has been a rapid increase in the number of cases recorded (Fig. 4.1). In 2005, the largest outbreak of salmonellosis linked to mung bean sprouts occurred within Ontario (Table 4.1). The implicated *Salmonella* serovar was Enteritidis, which is more commonly associated with poultry and raw eggs. How the *S.* Enteritidis strain became associated with the mung beans sprouts remains open to speculation although one major sprout producer within the province was targeted as the cause of the outbreak. The inability to trace human pathogens implicated in fresh produce foodborne illness outbreaks back to the original source is not uncommon and the ''smoking gun'' is rarely found. This can be attributed to the relatively short shelf life of fresh produce, which is often discarded by the time an outbreak is identified. The lack of traceability of produce is a further contributing factor that makes identification of specific sources problematic. However, the 2006 *E. coli* O157:H7 outbreak linked to baby spinach America was unique in that the strain of the pathogen was recovered from the infected people, spinach within unopened bags, and the farm where the crop was cultivated (Cooley *et al.*, 2007). The route of spinach contamination was considered to be through the transfer of *E. coli* O157:H7 from a cattle ranch near the field via infected wild pigs that found access to the crop through a broken fence. Yet, it is noteworthy that a survey of the Salinas valley in the summer of 2006 found a high prevalence of *E. coli* O157:H7 within the area, suggesting the actual route could have been via contaminated irrigation water (Cooley *et al.*, 2007).

Other notably outbreaks linked to fresh produce occurring in 2006 included lettuce, sprouts, cantaloupes, and *Clostridium botulinum* linked

to pasteurized carrot juice (Doyle and Erickson, 2008) (Table 4.1). The latter was of specific interest given that the neurotoxin levels detected in contaminated product were the highest ever recorded (FDA, 2006). As with the majority of fresh produce outbreaks the exact sequence of events that led to the six cases of botulism within the United States and Canada remains unexplained.

One of the largest foodborne illness outbreaks linked to fresh produce occurred primarily within the southern states of the US in 2008. The initial cause of the *Salmonella* Saintpaul outbreak was identified as tomatoes (Centers for Disease Control and Prevention (CDC), 2008; Lang, 2008). However, the failure to recover the *Salmonella* serovar from tomatoes shifted focus to peppers and cilantro (common ingredients of salsa) from Mexico. The outbreak lasted over 80 days and resulted in over 1400 cases being recorded (CDC, 2008). The actual number of cases is probably 10–30 times this figure given that the majority of illness outbreaks go unrecorded. Despite the number of cases involved in the *Salmonella* Saintpaul outbreak, no specific source or ''smoking gun'' was identified. However, the outbreak, like those within recent years, highlighted key deficiencies within the fresh-cut chain. Specifically:

- Inability to control the dissemination of human pathogens within the environment;
- Failure of post-harvest interventions to remove field acquired contamination;
- Lack of traceability to track contaminated produce back to the source.

The knowledge gaps associated with the microbiological safety within the fresh produce chain are significant. Despite the large body of research devoted to fresh produce, there still remains much to be known about the survival of pathogens within the environment and nature of interactions with growing plants. To understand the nature of human pathogen interactions with plants and survival in the environment, it is informative to provide a brief overview of the characteristics of those implicated in the majority of recorded outbreaks (Table 4.2).

III. CHARACTERISTICS OF PATHOGENS RECOVERED FROM SALAD VEGETABLES

A. Pathogenic *E. coli*

Nonpathogenic (generic) *E. coli* is a normal inhabitant of the gastrointestinal tract of humans and animals. However, some *E. coli* strains have now acquired virulence factors enabling them to cause disease of the gastrointestinal, urinary, or central nervous system. Pathogenic *E. coli* can be

TABLE 4.2 List of pathogenic bacteria and symptoms

Pathogen	Incubation period	Infectious dose and symptoms	Significant sources
Bacterial pathogens			
Aeromonas hydrophilia	Unknown, symptoms can last for several weeks	Gastroenteritis, septicemia, cellulitis, colitis, and meningitis Dose: Unknown but thought to be high ($>10^9$ cfu)	Water, sewage
Bacillus cereus	6–15 h diarrheal type 0.5–6 h emetic	Diarrheal type: watery diarrhea, abdominal cramps Emitic: vomiting, occasional abdominal cramps and/or diarrhea. Dose $>10^6$ cfu	Soil, starchy grains
Campylobacter jejuni	2–5 days	Diarrhea which may be watery or sticky and can contain blood. Guillain–Barre syndrome. Dose: >500 cfu	Manure especially derived from poultry production. Raw poultry
Clostridium botulinum	18–36 h	Neurotoxin affects nervous system leading to lethargy, weakness and breathing difficulty. LD_{50} 3 ng/kg	Soil, sediments, water
Escherichia coli O157:H7	24–48 h	Severe abdominal pain and diarrhea which is initially watery but becomes grossly bloody. Hemorrhagic colitis. > 100 cells	Manure from ruminants, sewage, raw beef
Listeria monocytogenes	1–90 weeks	Flu-like symptoms that may develop into septicemia, meningitis and encephalitis. Still birth or abortion in pregnant women. $>10^4$	Manure, sewage, soil, silage
Salmonella	24–48 h	Nausea, vomiting, abdominal cramps, diarrhea, fever, and headache. $>10^3$	Manure, soil, wild and domestic animals, sewage. Raw meat especially poultry

(*continued*)

TABLE 4.2 (*continued*)

Pathogen	Incubation period	Infectious dose and symptoms	Significant sources
Shigella sonnie	12–50 h	Abdominal pain; cramps; diarrhea; fever; vomiting; blood, pus, or mucus in stools; tenesmus >10 cells	Manure, sewage
Enteric viruses			
Norwalk Like Virus (Norovirus)	24–48 h	Nausea, vomiting, diarrhea, and abdominal pain	
Hepatits A	10–50 days	Sudden onset of fever, malaise, nausea, anorexia, and abdominal discomfort, followed by jaundice Dose: >20 virons	Sewage, infected food handlers and water
		Enteric protozoan	
Cryptosporidium parvum	2–4 days	Severe watery diarrhea Dose: >10 cells	Domestic animals, manure, sewage, infected food handlers and water
Cyclospora cayetanensis	7 days	Diarrhea which can last up to 6 weeks	Sewage and water
Giardia lamblia	7 days	Diarrhea which can last 1–2 weeks Dose: >1 cysts	Sewage, manure, wild and domestic animals, infected food handlers and water

Adapted from FDA Bad Bug Book (2008).

subdivided into five different categories based on the type of clinical condition they cause although all share common linkages.

All pathogenic *E. coli* stains follow a similar strategy of infection by colonizing the intestinal mucosal cells. The mode in which illness occurs varies between the different pathogenic *E. coli* types. ETEC and EaggEC produce enterotoxin, EIEC invades the epithelial cells with EPEC and EHEC adhering to the cell and modifying cellular activity.

Although all pathogenic *E. coli* represent a significant health risk, those belonging to the EHEC group are of most concern, especially *E. coli* O157:H7 (Weiner and Osek, 2007). The reason for the high virulence of EHEC is through the production of Shiga-like toxins (verotoxin or verocytotoxin). The genes for Shiga toxin are believed to have been horizontally transferred to *E. coli* from *Shigella* via bacteiophage. There are two toxins (encoded by *Stx 1* and *Stx 2*) that act by cleaving a single adenine residue from 28S rRNA belonging to the ribosomal subunit resulting in the shutdown of protein synthesis. The kidney is rich in receptors for attachment of *E. coli* O157:H7 and consequently toxicoinfection by the bacterium can be accompanied by renal failure (HUS syndrome) (Table 4.2).

Although *E. coli* O157:H7 is considered the most significant EHEC strain, it must be noted that other non-O157 Shiga-toxin producing types such as O111, O145, O113, O103, O91, O26, and O104 also exist (Bower, 1999). Collectively, all *E. coli* possessing toxin genes are categorized as Shiga-toxin *E. coli* or STEC. However, the presence of *stx* genes is only one of several virulence factors required to cause illness (McNally *et al.*, 2001). It is interesting to note that *E. coli* O157 and non-O157 serotypes associated with animals contain only half the virulent factors compared to those of clinical isolates (Johnson *et al.*, 2004). Therefore, the most virulent STEC have a tendency to be harbored by humans or introduced to animals in contact with sewage (Johnson *et al.*, 2004).

The main source of *E. coli* O157:H7 is from the manure of ruminants (cattle, sheep) and sewage (Chase-Topping *et al.*, 2008). Other livestock and wildlife have lower frequency of carriage. Although the estimates of STEC vary seasonally, and between herds, approximately 2–100% of cattle harbor *E. coli* O157:H7 (Hancock *et al.*, 1997). In a 12-month abattoir study in Great Britain, Milnes *et al.* (2008) determined the fecal carriage of STEC O157 to be 4.7% in cattle, 0.7% in sheep, and 0.3% in swine. Conversely, in another British study, Hutchison *et al.* (2005) isolated *E. coli* O157:H7 in 13% of fresh cattle manure, 21% of fresh sheep manure, and 12% in fresh swine manure.

ETEC, EIEC, and EAggEC have been previously recovered from contaminated vegetables (Robins-Browne, 2007; Scavia *et al.*, 2008) and are a major cause of diarrhea, especially in infants. The three groups of pathogenic *E. coli* can be water or foodborne although typically transmitted through person-to-person contact. EPEC, a further type of pathogenic

E. coli, is almost exclusively transferred via person-to-person contact although it has also been implicated in sporadic cases of foodborne illness (Ochoa *et al.*, 2008).

B. *Shigella*

Shigella sonnei has been implicated in several vegetable related foodborne illness (Table 4.1) outbreaks although it is normally associated with person-to-person contact (Solodovnikov *et al.*, 2008). Although *E. coli* O157:H7 and *Shigella* share pathological traits, the latter is less tolerant to environmental stress (Islam *et al.*, 1996). Therefore, in the majority of cases, infected food workers are considered the primary source of *Shigella*. However, outbreaks of foodborne illness associated with lettuce contaminated at preharvest with *Shigella* have occurred. The most notable was an outbreak involving contaminated iceberg lettuce imported from Spain into the United Kingdom, Norway, and Sweden. Subsequent investigations identified that irrigation water contaminated with human sewage was the source of the pathogen (Kapperud *et al.*, 1995).

C. *Salmonella*

The genus *Salmonella* includes over 2700 serovars, 200 of which are commonly associated with human illness with *S*. Typhimurium and *S*. Enteritidis being the most prevalent (Franz and van Bruggen, 2008). *Salmonella* is carried within the gastrointestinal tract of wild animals, poultry, pigs, and humans. However, *Salmonella* recovered from vegetables typically belong to less common serotype groups, for example Newport or Montevideo (Franz and van Bruggen, 2008).

There is concern with regard to the distribution of multidrug-resistant *Salmonella* within the food chain. It is commonly believed that the use of antibiotics as animal growth promoters has led to the prevalence of resistant serovars. Yet, through studying the epidemiology of *Salmonella*, it has become evident that the overprescription of antibiotics along with misuse (e.g., failure to complete a course of the drug) has played a significant role in the emergence of resistant strains (Kelly *et al.*, 2004). The possible use of antibiotics to suppress plant pathogens has been considered as a possible route by which *Salmonella* and other human pathogens can acquire resistance. Although this may seem unlikely, it is interesting to note that streptogramin-resistant *Enterobacter faecium* has been previously isolated from bean sprouts (Snary *et al.*, 2004).

Similar to *E. coli*, the main transmission route of *Salmonella* to vegetables is through fecal contamination, cross-contamination and food handling. In a 12-month abattoir study, Milnes *et al.* (2007) determined the fecal carriage of *Salmonella* to be 23.4% in swine 1.4% in cattle, and

1.1% in sheep. Hutchison *et al.* (2004) isolated *Salmonella* from 8% of fresh cattle manure, 8% of swine manure, 18% of poultry manure, and 8% of sheep manure; levels in stored manure were lower.

Salmonella have been isolated from a broad range vegetables especially sprouted seeds (Brandl, 2006; Johnston *et al.*, 2005). An interesting feature of *Salmonella* associated with vegetables (and other environmental sources) is the tendency to have low virulence compared to those isolated from clinical sources (Herikstad *et al.*, 2002; Olsen *et al.*, 2001; Sivapalasingam *et al.*, 2004). Evidence is accumulating to suggest that genes present within *Salmonella* enhance the survival of the pathogen outside the host environment. Significantly, mutants of *Salmonella* lacking such genes have higher virulence than their parent strain (Winfield and Groisman, 2004). Therefore, a number of *Salmonella* appear to have enhanced their survival in the environment at the expense of virulence. However, this does not imply of course that *Salmonella* associated with fresh produce represents a low risk.

D. Campylobacter

Campylobacter jejuni is a normal commensal of the gastrointestinal tract of poultry, pigs, and cattle. In a 12-month abattoir study, Milnes *et al.* (2007) determined the fecal carriage of thermophilic *Campylobacter* to be 54.6% in cattle, 43.8% in sheep, and 69.3% in swine. Hutchison *et al.* (2004) isolated *Campylobacter* from 13% of fresh cattle manure, 14% of swine manure, 19% of fresh poultry manure, and 21% of fresh sheep manure; levels in stored manure were significantly lower. Human carriers also represent significant vehicle by which the pathogen can be transferred to foods.

Campylobacter is notoriously fastidious and has very specific growth conditions. The bacterium can survive for short periods outside the host environment but not to the same extent as *Salmonella* and *E. coli* (Alter and Scherer, 2006; Garenaux *et al.*, 2008; Mihaljevic *et al.*, 2007). However, despite such fragility, *C. jejuni*, and to a lesser extent *Campylobacter coli*, has been the main cause of gastroenteritis for several years (Janssen *et al.*, 2008). This is likely due to the low infectious dose (<500 cells) required to cause symptoms in susceptible hosts and the high carriage rate in livestock (Ozcakir, 2007).

C. jejuni invade and become established in epithelial cells of the lower intestine whereupon a cholera-like toxin is secreted. The main symptom associated with the disease is perfuse diarrhea that can last between 2 and 14 days although is rarely life threatening. There has been an increase in the recovery of antibiotic-resistant *C. jejuni* from human isolates although not from animals (Pumbwe and Piddock, 2004).

C. jejuni have been recovered from vegetables especially root crops (Brandl *et al.*, 2004). However, evidence to date suggests that the main source of *Campylobacter* recovered from vegetables occurs via cross-contamination events in food service outlets and the domestic environment.

E. *Listeria monocytogenes*

Unlike typical enteric bacteria, *L. monoytogenes* has adapted to survive in both the host and nonhost environment. Because *L. monocytogenes* is widely distributed in nature, the pathogen is a common contaminant of vegetables, especially root crops (Embil *et al.*, 1984; Lianou and Sofos, 2007; Swaminathan and Gerner-Smidt, 2007). Hutchison *et al.* (2004) isolated *Listeria* from approximately 30% of fresh cattle manure, 20% of swine manure, 19% of poultry manure, and 29% of sheep manure. The latter is of relevance given that the first identified foodborne listeriosis outbreak was linked to coleslaw (cabbage) fertilized with uncomposted sheep manure (Embil *et al.*, 1984).

The virulence of *L. monocytogenes* is often under estimated considering the pathogen causes serious illness that frequently results in death (Drevets and Bronze, 2008). Similar to other human pathogens, the virulence of *L. monocytogenes* that can tolerate environmental stress is lower than those recovered from clinical cases of listeriosis (Chan *et al.*, 2007).

F. *Aeromonas hydrophila*

Aeromonas are widely distributed in the environment especially water but can occur in human feces (Campo *et al.*, 2001). Two distinct types of gastroenteritis have been associated with *A. hydrophila*, a cholera-like illness with a watery diarrhea and a dysenteric illness characterized by loose stools containing blood and mucus (Pund and Theegarten, 2008). The ability of *Aeromonas* to cause illness depends on the presence and expression of virulence factors. Although the bacterium is frequently recovered from water in high densities (10^5–10^9/100 ml), very few strains have the capacity to cause illness in humans. This has been related to the lack of complete virulence factors and high tolerance of the host (Chifiriuc *et al.*, 2007; Pund and Theegarten, 2008). Therefore, although widely distributed on vegetables, the pathogen is more significant in ready-to-eat foods such as meat and soft-cheeses.

G. Endospore-forming bacteria

Spores of *Cl. botulinum* and *Cl. perfringens* can be found both in soil and vegetables. Because clostridia are obligate anaerobes, their growth is restricted on fresh produce stored aerobically or under hyperoxygenated modified atmosphere. However, there is a trend to preserve vegetables in oil which is more conducive to the growth of clostridia (Barker *et al.*, 2005; Carlin and Peck, 1996; Peck, 2006). Indeed, there have been several botulism outbreaks linked to vegetable-in-oil products (Lohse *et al.*, 2003).

Bacillus cereus is an aerobic spore-forming bacterium that is widely distributed in soil and on plant material. Therefore, its occurrence of

vegetables is not uncommon especially with regard to leafy vegetables and sprouts (Joo *et al.*, 2004; Kim *et al.*, 2004).

H. Enteric viruses

Enteric viruses only reproduce within the human host and all follow the fecal–oral route of transmission (Girard *et al.*, 2006). The most significant characteristic of enteric viruses is the low infectious dose to cause illness (<20 virons) and the ease by which they can be transferred from person to person (Mattison *et al.*, 2007). Enteric viruses are also very stable with resistances to environmental stresses comparable to those associated with bacterial endospores (Mattison *et al.*, 2007).

The majority of foodborne illness associated with enteric viruses are short-lived and not life threatening (Kurdziel *et al.*, 2001). Tracing sources of enteric viruses is problematic due to the lack of routine detection techniques combined with under-reporting of outbreaks (Kurdziel *et al.*, 2001).

The majority of outbreaks linked to enteric viruses is typically caused by person-to-person contact although fresh produce can also be a significant vehicle, especially with regards to soft fruit which are handled and rarely washed prior to consumption (Koopmans, 2008). Both hepatitis A and Norwalk-like viruses (NLV) have been implicated in cases of foodborne illness associated with contaminated vegetables (Koopmans, 2008). In such outbreaks, the crops had been directly exposed to sewage or had been handled by infected workers (Holtby *et al.*, 2001; Long *et al.*, 2002).

A high profile hepatitis A outbreak associated with green salad onions was reported in the United States in 2003 (Chancellor *et al.*, 2006). The initial outbreaks were centered on a restaurant in Pennsylvania that resulted in 575 cases of hepatitis A and 1 death (Vale, 2005). Although the restaurant was initially identified as the source of the outbreak, subsequent investigation linked other cases in Tennessee and Georgia. Further inspection of the farm in Mexico was later identified as the most likely source of the virus (Vale, 2005).

Interestingly, the persistence of viruses such as polio has been shown to be dependent on the vegetable type. When introduced onto lettuce or cabbage, a 1 log reduction in polio virus was observed over 8 days. In contrast, viruses introduced onto green onions remained stable for over 14 days (Kurdziel *et al.*, 2001). The underlying factors associated with the persistence of enteric viruses on fresh produce remain to be elucidated.

I. Human pathogenic protozoa

Human pathogenic protozoa such as *Giardia*, *Entamoeba*, *Toxoplasma*, *Sarcocystis*, *Isopora*, *Cryptosporidium*, *Eimeria*, and *Cyclospora* can be transferred via fecally contaminated water or vegetables (Armon *et al.*, 2002).

Similar to enteric viruses, protozoa require a suitable host for replication but can persist within nonhost environments for significant time periods (Sidhu and Toze, 2009). The main source of human protozoan is from direct contact with humans although foodborne sources can also represent a significant vehicle (Sidhu and Toze, 2009; Thompson *et al.*, 2008). All of these human pathogenic protozoa cause diarrhea-like symptoms except *Toxoplasma*, which causes fetal damage and glandular fever-like syndrome (Dumetre and Darde, 2003).

The prevalence of *Cryptosporidium* and *Giardia* from human, agricultural, or wildlife sources was examined by Heitman *et al.* (2002). The researchers found that while sewage effluent had the highest prevalence of the parasites by far, the greatest concentrations were detected in cattle feces. However, direct linkages, using molecular techniques, between cryptosporidiosos outbreaks and contamination from livestock sources are limited. *Cryptosporidium* has been isolated from 5% of fresh cattle manure, 13.5% of swine manure, and 29% of sheep manure. *Giardia* prevalence is similar to that of *Cryptosporidium* being found in 4%, cattle, 2% swine, and 21% sheep manure samples (Hutchison *et al.*, 2004; Thompson *et al.*, 2005).

IV. TRANSMISSION OF HUMAN PATHOGENS IN MANURE, SOIL, AND WATER TO THE VEGETABLE PRODUCTION CHAIN

Enteric pathogens have to be introduced into the production chain at some point in order to contaminate fresh produce. Direct fecal contamination of vegetables just prior to consumption represents the greatest risk (Gorny, 2002; Mukherjee *et al.*, 2007). However, other sources of contamination such as manure amended soil and irrigation water are more commonly encountered (Hutchison *et al.*, 2008; Islam *et al.*, 2004).

Enteric pathogens on growing vegetables are generally believed to be in survival mode as opposed to actively growing. Previously, it has been considered that enteric bacteria such as *E. coli* only survive for 2–3 days after being excreted by the animal host. However, such generalizations are inappropriate considering that *E. coli* populations within the gastrointestinal tract of animals can consist of over 1000 distinct genotypes (Gordon *et al.*, 2002). More significantly, the *E. coli* associated with animals have a broad range of survival abilities within nonhost environments. It has also been found that those genotypes/strains that dominate the enteric environment have relatively poor survival outside the host environment (Whittam, 1989). Evidence obtained to date would also suggest that strains of *Salmonella* and *E. coli* O157:H7 also exhibit a range of survival abilities during the transition from the gastrointestinal tract to

the environment (Winfield and Groisman, 2003, 2004). This has naturally complicated studies attempting to determine the relative survival of enteric pathogens in the environment. In this respect it is likely that many studies previously performed (especially with laboratory strains) underestimated the tolerance of pathogens to environmental stresses hence their persistence in manure, soil and water.

A. Manure and biosolids

Farm and sewage effluents represent the most significant source of human pathogens recovered in water, soil and hence, vegetables (Baloda *et al.*, 2001; Beuchat and Ryu, 1997; Davies *et al.*, 2008; McGrath *et al.*, 1995; Rideout and Teschke, 2004). Manure is predominantly used in organic cultivation systems but less so by conventional growers (USDA, 2001). Although organic produce is thought to represent a significance risk with regards to carriage of enteric pathogens, no data have been reported to confirm this view (Loncarevic *et al.*, 2005). The application of untreated manure or sewage to growing crops is a direct route by which vegetables can be contaminated (Culley and Barnett, 1984; Franz and van Bruggen, 2008). For example, Cieslak *et al.* (1993) isolated *E. coli* O157:H7 from lettuce cultivated in a garden in which the soil was amended with fresh manure. Under normal conditions the direct contact of manure with vegetables should not occur since, either a treatment step is applied prior to disposal of effluent into soil or water, or there is a significant time interval between application and crop production. Often regulatory agencies will identify an appropriate minimum time delay between manure application and fruit or vegetable harvest. For example, Canadian regulations specify 3 months for tree fruits and grapes, 15 months for small fruits, and 12 months for vegetables. The USDA ''organic production and handling requirements'' specify that, unless composted, raw animal manure must be incorporated into the soil not less than 120 days prior to harvest of a product whose edible portion has direct contact with the soil surface or soil particles, or 90 days if there is no direct contact (USDA, 2005).

Manure and sewage waste can undergo a variety of treatments such as composting, aerobic and anaerobic digestion, alkaline stabilization, conditioning, dewatering, and heat drying. The Environmental Protection Agency specify that biosolids derived from manure treatment destined for general fertilizer must have fecal coliform counts <1000 cfu/g, *Salmonella* < 4 cfu/g, and enteric viruses at <4 plaque-forming units/g biosolids (Mechie *et al.*, 1997). Similarly, Canadian regulations specify fecal coliforms <1000 MPN/g dry weight of total solids or no *Salmonella* with a detection level <3 MPN/4 g dry weight total solids (CCME, 2005).

In the main, proper manure sewage/manure treatment (e.g., composting) can be sufficient to inactivate human pathogens. However, the

treatment required to assure adequate reduction of enteric pathogens in manure remains a debatable issue. Current treatment regimes tend to be based on studies that evaluated the survival of endogenous or artificially introduced human pathogens in manure held under a narrow range of conditions. Therefore, the reported persistence of human pathogens in manure tends to vary significantly (Cote and Quessy, 2005). However, it is widely accepted that the survival of bacterial and viral human pathogens is dependent on temperature, aeration and holding time, dry matter content, pH, and bacterial concentration (Ingram and Millner, 2007; Leifert *et al.*, 2008; Mannion *et al.*, 2007; Semenov *et al.*, 2007; You *et al.*, 2006).

E. coli O157:H7 can survive in high moisture content bovine manure for over 70 days at 5 °C that compared to 49 days at 30 °C (Semenov *et al.*, 2007). The persistence of *Salmonella* in manure is also favored under low temperature and high moisture conditions (Mannion *et al.*, 2007). However, survival of both pathogens in manure slurry is restricted to a maximum of 10 days (Cote *et al.*, 2006). Laboratory studies on *E. coli* O157:H7 and *S.* Typhimurium in cow manure and cow slurry indicated that death rate is a function of temperature and depth in the storage container (Himathongkham *et al.*, 1999). Decimal reduction times varied from 6 days to 3 weeks, with the decline being fastest at 37 °C compared to the colder temperatures. The authors suggested that this data should enable predictions of storage conditions that would lead to predetermined levels of reduction of the two pathogens.

Several studies of inoculated pathogens in stored manure have attempted to determine survival rates. Hutchison *et al.* (2005) examined the declines of inoculated *Salmonella*, *E. coli*, *Campylobacter*, *Listeria*, and *Cryptosporidium* in summer and winter storage in 35,000 L tanks of fresh waste (slurries, dirty waters). *D*-values (days for 1 − log cycle decrease) ranged from 6 to 44 days, and generally in the order *Campylobacter* (mean of 10.7 days) < *Listeria* (13.9 days) < *Salmonella* (14.6 days) < *E. coli* O157 < *Cryptosporidium parvum* (232 days). Interestingly, mean summer and winter decline rates were similar, and slurry dry matter was not found to be significant. The authors found that less than 6 months passive storage was sufficient to reduce bacterial populations below detectable levels, but insufficient for significant reduction of *C. parvum*. In a similar study, Nicholson *et al.* (2005) examined solid and liquid manures mixed with *E. coli* O157:H7, *Listeria*, *Campylobacter*, and *Salmonella* (dairy and pig solid, broiler litter, dairy slurry at 7% and 2% dry matter, and dirty water). They found that survival in solids varied from 2 to 32 days, and was lowest in turned and unturned piles when the temperature exceeded 50 °C. Survival was found to be in the order *Campylobacter* < *Listeria* < *Salmonella* < *E. coli* in solid manure, and *Salmonella* < *Listeria* = *E. coli* < *Campylobacter* in liquid manure and dirty water.

Treatment of manure piles through mixing or composting can increase pathogen kill-off. Nicholsen *et al.* (2005) demonstrated survival of *E. coli* O157:H7 in sheep manure in aerated piles up to 4 months, but in static piles up to 21 months. In aerated bovine manure piles, the organism persisted for 47 days. However, Fleming and MacAlpine (2002) did establish rapid, temperature-related *E. coli* and *Salmonella* die-off during composting of liquid swine manure mixed with various materials. Composting temperatures that exceed 55 °C for 3 days are generally considered to eliminate most pathogens (Duffy, 2003; Grewal *et al.*, 2006; Turner, 2002; Turner *et al.*, 2005).

C. jejuni persistence in manure has traditionally thought to be comparatively low, being 3 days in cattle manure and 2 days in sewage (Hutchison *et al.*, 2005). However, in field studies, *Campylobacter* has been detected in undisturbed liquid swine manure held in tanks for several weeks, and storage under winter conditions (frozen surface) prolongs survival (Huber, unpublished results).

Unlike vegetative cells, enteric virus can persist in sewage for up to 4 months under low temperatures and high moisture (Divizia *et al.*, 2008; Gino *et al.*, 2007).

B. Irrigation water

Irrigation water is susceptible from direct contamination from sewage or manure spills. In the course of heavy rainfall, contamination associated with manure heaps can leach into water courses (Heaton and Jones, 2008; Lazarova and Savoys, 2004; Ottoson and Stenstrom, 2003). The level of contamination from these sources varies significantly. General population levels of *E. coli* in variety of potential contamination sources are presented in Table 4.3.

Irrigation water used in crop production represents one of the most significant sources of contamination in fresh produce production. Contamination from manure heaps can readily be transferred to the water course via run off and subsequently disseminated over large distances via streams or rivers. When contaminated, water is used to irrigate crops, human pathogens can be directly transferred to plant tissue and persist through to harvest.

Salad crops irrigated with water contaminated with sewage was responsible for numerous cases of typhoid fever and hepatitis A in Santiago, Chile. Lettuce irrigated with contaminated water can accumulate *E. coli* O157:H7 over repeated exposures (Franz and van Bruggen, 2008). Lettuce plants exposed to contamination 7 days or less prior to harvest represent a greater risk than contaminated irrigation water introduced early in the cultivation period (Solomon *et al.*, 2002). General Canadian guidelines require that irrigation water for food crops contain

TABLE 4.3 Levels of *Escherichia coli* encountered in different manure, effluent and waste water

Contamination source	Level of *E. coli* in source (log_{10} cfu/100 ml)	Data source
Liquid manure	5–7	Huber (unpublished data)
Runoff from manure piles	4–6	Huber (unpublished data)
Raw sewage	6–8	Servais *et al.* (2007)
Dairy wash water	0–4	Huber (unpublished data)
Fruit and vegetable wash water	0–3	OMAFRA (unpublished data)
Streams through grazing land	4	Servais *et al.* (2007)
Surface water through cropland	3	Servais *et al.* (2007)

less than 100 cfu fecal coliforms/100 ml (CCME, 2005); some provincial requirements may be lower (e.g., B.C. regulations for *E. coli* < 77 cfu/100 ml).

Recycling of municipal waste water (gray water) for irrigation purposes have been implemented in several countries such as Australia, Germany, Israel, Spain, Holland, and the USA. However, studies have illustrated that the practice of using recycled water may increase the risk of introducing human pathogens (Gross *et al.*, 2007; O'Toole *et al.*, 2008). For example, onions and garlic cultivated with treated municipal waste water harbored unacceptable levels of *Salmonella* and *E. coli* at harvest (Fasciolo *et al.*, 2002). Regulations for recycled water vary from average nondetectable to 200 cfu/100 ml depending on US state and crop (Table 4.4). For food crops, recycled water has had to undergo tertiary treatment, usually involving disinfection, again depending on the state. Regulations for other pathogens are not specified, but Florida requires monitoring for *Cryptosporidium* and *Giardia* on a regular basis.

The method of applying irrigation water can also enhance the introduction of human pathogens to growing vegetables (FDA, 1998). The various irrigation modes used for vegetables include gravity (flood) irrigation, spray irrigation, drip/trickle irrigation, and subirrigation (FDA, 1998). Many factors, such as water availability and cost, soil type, slope, depth of water table, economics, and cropping rotations, determine the mode of irrigation rather than food safety issues. Flood and spray irrigation represent the greatest risk as any contamination within the water is directly deposited onto the edible leaves of crops (FDA, 1998).

TABLE 4.4 Recycled water standards in different states with the US

	Arizona	California	Florida	Hawaii	Nevada	Texas	Washington
Treatment	Secondary treatment, filtration and disinfection	Oxidized, coagulated, filtered and disinfected	Secondary treatment, filtration and high-level disinfection	Oxidized, filtered and disinfected	Secondary treatment and disinfection	NS	Oxidized, coagulated, filtered and disinfected
BOD_5	NS	NS	20 mg/l	NS	30 mg/l	5 mg/l	30 mg/l
TSS	NS	NS	5 mg/l	NS	NS	NS	30 mg/l
Turbidity (NTU)	2–5 Fecal	2–5 Total	NS Fecal	2 Fecal	NS Fecal	3 Fecal	5 Total
Coliform average	ND	2.2/100 ml	75% below level of detection	2.2/100 ml	200/100 ml	20/100 ml	2.2/100 ml
Coliform maximum	23/100 ml	23/100 ml	25/100 ml	23/100 ml	400/100 ml	75/100 ml	23/100 ml

Source: *Environmental Protection Agency (2004).*

There have been numerous studies performed to determine the persistence of human pathogens within irrigation water (Ajwa *et al.*, 2002; Armon *et al.*, 2002; Bastos *et al.*, 2008; Fasciolo *et al.*, 2002; Islam *et al.*, 2004; Ottoson and Stenstrom, 2003) (Table 4.5). There are difficulties in relating the relative persistence of enteric pathogens in water since no standardized experimental protocol is followed. In addition, the occurrence of viable but nonculturable (VBNC) populations of pathogens can further complicate survival values (Lee *et al.*, 2007; Liu *et al.*, 2008; Ozcakir, 2007). For example, *Salmonella* species (including *S.* Typhimurium DT 104) introduced into autoclaved river water decreased from 8 log to 5 log cfu/ml over a 45-day period at 23 °C (Santo Domingo *et al.*, 2000). However, when the VBNC cells were examined, less than 1 log of reduction was observed. Lower temperatures also extended the persistence of enteric pathogens in water (Santo Domingo *et al.*, 2000). In general, the persistence of *Salmonella* is greater than that of *E. coli* O157:H7 which in turn persists for longer times than *Campylobacter* (Krampitz and Hollander, 1999; Ottoson and Stenstrom, 2003).

Survival studies of enteric pathogens are typically performed in sterilized (autoclaved or filtered) water samples. Although this facilitates enumeration of the introduced pathogen, it does not provide an assessment of survival in natural environments. In this respect it is interesting to note that survival of enteric pathogens in nonsterile water is significantly shorter due to the activity of protozoan (Artiz and Killham, 2002). However, protozoan can form protective niches for enteric pathogens, thereby enhancing persistence under certain conditions (Wang *et al.*, 2005).

The persistence of *E. coli* O157:H7 has also been found to vary depending on the source of water. Artiz and Killhem (2002) evaluated the survival of *E. coli* O157:H7 in water sourced from four different wells. The authors reported that in two water samples, *E. coli* O157 introduced at levels of 10^7 cfu/ml were reduced to below the level of detection within 10 days regardless of being suspended in sterile or nonsterile water. Although not confirmed, this low level of persistence was attributed to the presence of antimicrobial ions such as copper. The study underlines the difficulties encountered when attempting to predict the survival of enteric pathogens in water.

Giardia cysts persist for a shorter period in irrigation water compared to *Cryptosporidium* oocysts (Karim *et al.*, 2004; Skraber *et al.*, 2007). Temperatures as low as −4 °C inactivate *Giardia* cysts in water while *Cryptosporidium* oocysts remained viable for >12 weeks at 4 °C (Karim *et al.*, 2004). At 25 °C, *Giardia* cysts were inactivated in water within 2 weeks but *Cryptosporidium* oocysts survived for >10 weeks. Factors affecting the survival of pathogens in water have been reviewed by Bichai *et al.* (2008).

TABLE 4.5 Survival of human pathogens in different water sources

Pathogen	Notes	Temperature (°C)	Survival	Reference
E. coli O157	Sterile municipal water	8	91 days	Wang and Doyle (1998)
	Sterile municipal water	25	49 days	Wang and Doyle (1998)
	Sterile well water	15	1 log reduction in 70 days	Artiz and Killhem (2002)
	Well water	15	65 days	Artiz and Killhem (2002)
	Sterile well water	15	10 days	Artiz and Killhem (2002)
Salmonella	Sterile municipal water	23	2 log reduction after 45 days	Santo-Domingo *et al.* (2000)
	River water	23	3 log reduction after 45 days	Santo-Domingo *et al.* (2000)
	Sterile well water	18	152 days	Mitscherlich and Marth (1984)
Campylobacter	Sterile municipal water	4	8–28 days	Terzieva and McFeters (1991)
	Sterile municipal water	37	22 h	Terzieva and McFeters (1991)
Yersinia enterocolitica	Sterile spring water	4	446 days	Karapinar and Gonul (1991)
	River water	16	6 days	Chao *et al.* (1988)
	Groundwater	30	10 days	Chao *et al.* (1988)
Rotavirus	Groundwater	15	2 log reduction in 5 days	Gerba (1999)

C. Soil

Soil is a natural habitat for several human pathogens such as *B. cereus*, *Cl. botulinum*, and *Cl. perfringens*, *L. monocytogenes*, and *Aeromonas* (Waage *et al.*, 1999). The aforementioned bacteria have adapted to survival in soil with spores persisting for indefinite periods.

The persistence of enteric pathogens in soil is dependent on several factors. For example, the survival of *E. coli* is prolonged in clay soils where absorption of cells to the soil particles provides protection against protozoa (Lang and Smith, 2007; Mosaddeghi *et al.*, 2009; Wong *et al.*, 2008). *E. coli* O157:H7 has been reported to persist for 25 weeks in loam and clay soils, but for 8 weeks in sandy soils (Lang and Smith, 2007). Persistence of enteric pathogens is also extended in moist soils at cool temperatures (Lang and Smith, 2007). *Salmonella* has higher persistence in soil compared to *E. coli* O157:H7. When *S.* Typhimurium was inoculated at 8 $\log_{10}$ CFU/g into moist soil, stored at 20 °C, less than 2 log reductions in numbers were observed after 45 days (Guo *et al.*, 2002). However, under natural environmental conditions, *S.* Typhimurium introduced via hog manure only persisted for 14 days (Sengelov *et al.*, 2003). *Campylobacter* is less persistent compared to both *E. coli* O157 and *Salmonella* but nevertheless can be recovered 20 days after introduction into soil (Nicholson *et al.*, 2005).

The survival of common manure-derived pathogens in soils was reviewed by Nicholson *et al.* (2005), who concluded that maximum survival ranged from 45 to 100 days with an average log reduction of 1.94 days for *E. coli* O157, *L. monocytogenes*, *Salmonella*, and *C. jejuni.* The time for a 1 − log reduction in *C. parvum* levels, however, varied between 8 and 31 days.

In addition to stress, enteric bacteria also have to compete with the endogenous microflora to become established within the soil environment. It has hitherto been considered that enteric pathogens compete poorly for nutrients and are susceptible to inhibition by soil-borne bacteria. Indeed, *E. coli* O157 have been shown to decline more rapidly in manure-amended unautoclaved soils compared to autoclaved soil (Jiang *et al.*, 2002). However, Ibekwe *et al.* (2004) reported that introduction of *E. coli* O157:H7 into soil increases the diversity of microbial populations. This would suggest that enteric pathogens, rather than being integrated into soil microflora, can actually modify the microecology. Whether this effect enhances persistence has yet to be elucidated.

Agronomic practices can impact the survival of manure-derived pathogens in the soil. Generally, bacteria are thought to decline more rapidly when manure is left on the surface rather than incorporated into the soil immediately after application, presumably due to the elimination of drying conditions and exposure to UV at the soil surface (Diaz and

Schulze-Makuch, 2006). Huber (unpublished data) determined the decline of *E. coli* and *Clostridium* in soil amended with liquid swine manure. *E. coli* declined after 28 days, following a spring application, but was still detected 55 days after a fall application, while *Clostridium* remained detectable longer than 55 days after both spring and fall applications. Season and location played a role in the length of time pathogens and fecal indicators were detected, suggesting that soil type or environmental factors such as rainfall play a role in survival. Interestingly, *E. coli* was detected in tile drainage waters after rainfall up to 77 days after application of manure to the soil, suggesting that these organisms can persist and may mobilize into waterways after prolonged periods of time.

Enteric viruses can persist for up to 4 months in subsurface soil layers. In contrast, viruses on the surface are typically inactivated within days by the antimicrobial effects of UV (Gerba *et al.*, 2002; Moll and Vestal, 1992). Under heavy rainfall, viruses can be spread over wide areas (>150 m) especially when introduced into water courses (Santamaria and Toranzos, 2003). While *Giardia* is sensitive to freezing of soil, *Cryptosporidium* is more resistant. Mahdy et al. (2008) reported *Giardia* cysts in soil were inactivated after 7 days at −4 °C, but *Cryptosporidium* could survive for >12 weeks. However, persistence of both protozoa was reduced to 8 weeks at 4 °C and 4 weeks at 25 °C (Mahdy *et al.*, 2008). *Cryptosporidium* have been shown to be particularly sensitive to drying. Various studies have shown less than 5% viability following 4 h of air drying at room temperature (Anderson, 1986; Nasser *et al.*, 2007; Robertson *et al.*, 1992).

D. Transport of human pathogens within the environment

Movement of pathogens from manure storages or manure or biosolids-fertilized cropland to surface or groundwater can be a significant source of contamination of water that may be used for irrigation purposes (Berry *et al.*, 2007; Hill *et al.*, 2005; Muirhead *et al.*, 2006). This can occur as the result of over application of liquid manures or biosolids, but more commonly occurs in response to heavy rainfall shortly following manure or biosolids application (Muirhead *et al.*, 2006). Potential also exists for rainfall-induced surface transport from treated cropland directly to fruit and vegetable fields.

Astrom *et al.* (2007) compared the total coliform and *E. coli* contamination of surface runoff and tile effluent from two no-till field plots: one with surface-applied liquid dairy manure and a nonmanure-treated plot. These researchers found greater bacterial populations in surface and tile waters from the manure treated plot, but significant contamination occurred from the nonmanured plot attributed to bird and other wildlife feces. Soupir *et al.* (2006) examined the release and transport of bacteria from manure applied to pastureland. Using rainfall simulations, they

found that from 30% to >100% of the released bacteria were transported in overland flow to the edge of the field, depending on the manure type. Tyrrel and Quinton (2003) using soil flumes, found that incorporation of slurries reduced the transport of fecal coliform in overland flow.

A long-term Ontario study by Culley and Phillips (1982) examined the bacterial quality of runoff and subsurface discharge waters (total and fecal coliforms and fecal streptococci). They found that neither the rate nor application time affected the bacterial content of spring surface and subsurface discharge unless the manure was applied on frozen soil. In this case, spring snowmelt dominated runoff and significantly decreased discharge water quality, indicating survival over winter and subsequent transport to surface waters.

Application of manure onto tile drained agricultural fields creates significant potential for transportation of pathogens into source waters directly through tile drains, or by overland flow into surface waters. A number of studies have been conducted that address pathogen movement through tile drains (Lapen *et al.*, 2008). Dean and Foran (1992a,b) demonstrated contamination of tile water following liquid manure application in 8 out of 12 ''normal'' farm applications. Greco (2002) demonstrated loss of a marker *E. coli* to tile waters via preferential flow through macropores within hours of liquid manure application and a simulated rainfall. Similarly, Akhand *et al.* (2008) demonstrated liquid municipal biosolid and precipitation induced tile flow on silt (hence potential pathogens) loam soils. In both studies, flow to tile drains occurred within hours of significant rainfall and was dominated by macropore flow. The dissemination of bacteria into tile drains can occur within hours following manure application followed by a significant rainfall. However, the transport of *E. coli* can be reduced by pretillage of soil prior to manure application (Hubar, unpublished results).

Preventing contamination of irrigation water is problematic due to the open nature of animal production and problems associated with manure management. Nevertheless, monitoring the microbiological quality of water is a key intervention to reduce the risk of transferring contamination to fresh produce. Furthermore, when contamination is detected in water there is a need to rapidly identify the source and implement containment plans. Microbial source tracking (MST), which is a collection of chemical, physiological, and genetic methods, can differentiate or identify sources of fecal contamination (Nayak and Stewart-King, 2008; Parajuli *et al.*, 2009; Reischer *et al.*, 2008). MST methods are further divided into library-dependent and library-independent methods. Library-based methods require a database to be constructed using, for example, *E. coli*, collected from a diverse range of environmental, human, domestic, and wild animals (Santo Domingo *et al.*, 2007; Stoeckel and Harwood, 2007). The isolate derived from the water sample is then compared with profiles

within the database to identify the possible source of fecal contamination. The underlying basis principle of library-based methods is that stains of the target bacterium is specific to animal or human sources, and that the population is stable and has geographical structure (Barnes and Gordon, 2004; Gordon *et al.*, 2002). That is, strains, are specific to the host, the population is not turned over (i.e., database is valid over long time periods), and strains isolated within one area can be differentiated from those located in a different geographical location.

The fingerprint generated by MST can be physiological (e.g., Biolog) or more commonly using a genetic technique such as rep-polymerase chain reaction (Stoeckel and Harwood, 2007). Library-independent approaches are methods based on quantification of host-associated markers such as coliphage and *Bacteroidales*, or chemicals, such as caffeine or fecal sterols that signifies human, as opposed to animal sources (Stoeckel and Harwood, 2007).

There have been mixed reports on the success of MST to trace sources of fecal contamination (Harwood *et al.*, 2003; Myoda *et al.*, 2003; Stoeckel *et al.*, 2004). In the case of methods based on *E. coli*, the main limitations were with respect to the reliability of the database. Specifically, *E. coli* strains are not entirely host specific, their populations are highly diverse with low temporal stability and poor geographical structure (Barnes and Gordon, 2004; Davis and Gordon, 2002; Dixit *et al.*, 2004; Gordon, 2001; Gordon *et al.*, 2002).

It is generally understood that a multitiered approach, combining library-based and library-independent techniques is the most reliable way to identify fecal source pollution. A number of studies have used this combination of approaches to identify sources of contamination, in agriculturally contaminated watersheds has been undertaken and shown promise (Vogel *et al.*, 2007).

V. INTERACTION OF PATHOGENS WITH FRESH PRODUCE

A. Survival in the phyllosphere

The phyllosphere (or aerial) parts of plants represent a challenge for the survival of microbes. The exposure to high doses of UV, fluctuations in temperature, and relative humidity all compromise viability (Heaton and Jones, 2008; Whipps *et al.*, 2008). Bacteria (epiphytes) that exist within the phyllosphere have evolved specialized mechanisms to improve stress tolerance and nutrient acquisition. *Pseudomonas* spp. form the predominant bacterial population recovered on the leaves of plants (Brandl and Amundson, 2008; Lindow and Brandl, 2003). Epiphytic pseudomonad's produce fluorescent or pigmented compounds that afford protection to UV.

The hydrophobic waxy cuticle of plants can inhibit the movement and accessibility of nutrients to bacterial cells. However, biosurfactants produced by the majority of epiphytic *Pseudomonas* spp. decreases the water tension, enabling relatively free movement across the leaf surface to nutrient sources and natural openings such as stomata. *Pseudomonas* are also known to release a toxin called syringomycin that can produce holes in the plant cell membrane allowing access to intracellular nutrients without necessarily resulting in disease symptoms (Cao *et al.*, 2005).

The association of human pathogens with biofilms formed by resident epithytes is considered to enhance survival on leaf surfaces. It has been estimated that 10–40% of the total bacteria on the surface of parsley and broad-leaf endive are associated with biofilms (Lindow and Brandl, 2003). However, studies performed with *E. coli* O157:H7 or *Salmonella* would suggest that bacterial cells tend to aggregate between the grooves of epidermal cells rather than associate with biofilm structures (Warriner *et al.*, 2003a).

There have been relatively few studies with regard to the survival of human pathogens on the surface of leaves over long periods. However, studies using *C. jejuni*, *E. coli* O157:H7, and *Salmonella* would suggest that this is significantly lower compared to the rhizosphere (Brandl and Amundson, 2008; Brandl *et al.*, 2004). Nevertheless, as previously outlined, contamination of edible leaves immediately prior to harvest would represent a significant food safety hazard.

B. Colonization of the rhizosphere

The ability to utilize the nutrients released by seeds or roots is considered a prerequisite in becoming established in the rhizosphere of plants prior to internalization (Buyer *et al.*, 2002). In this respect, enteric pathogens, including *Campylobacter*, can actively grow on exudates released by plants (Brandl *et al.*, 2004; Gagliardi and Karns, 2000). There is also evidence that human pathogens have extended persistence within the environment by becoming integrated into the rhizosphere of plants. Gagliardi and Karns (2002) found that the presence of certain crops increased the persistence of *E. coli* O157:H7 in soil. In unplanted, fallow soils, *E. coli* O157:H7 persisted only for 25–41 days, but was found up to 92 and 96 days if alfalfa or rye plants were present, respectively. Ibeweke *et al.* (2004) also found that the presence of roots in contaminated soils increased concentrations of *E. coli* O157:H7. In this study, *E. coli* introduced through irrigation water was found to reach higher densities in rhizosphere soils than in nonrhizosphere soils (Ibeweke *et al.*, 2004). Bacterial populations in soil increased after the addition of plant material to soil; the bacterial population spiked and then fluctuated in a wave-like fashion that was not found to be the result of nitrogen shortages or pH (Ibeweke *et al.*, 2004). In contrast to

these results, the presence of maize roots did not affect survival of *E. coli* in soil (Bernstein *et al.*, 2007). Likewise, the presence of other legume crops other than alfalfa did not increase persistence of *E. coli* O157:H7 more than in fallow soils (Gagliardi and Karns, 2002).

C. Internalization of human pathogens in growing plants

The possibility of pathogens such as *E. coli* O157:H7 and *Salmonella* to become internalized into the vascular system of growing plants has received significant attention (Table 4.6). Once internalized, pathogens are protected from postharvest washing and cannot be readily removed or inactivated. It has been conclusively proved that human pathogens can enter stomata and cut edges of fresh produce (Seo and Frank, 1999; Takeuchi and Frank, 2000; Takeuchi *et al.*, 2000). Damage caused by spoilage bacteria/fungi can also enable human pathogens to enter the inner plant tissue and thereby become protected (Brandl, 2008). However, evidence that human pathogens can infiltrate the roots of intact growing plants and become established as an endophyte has yet to be demonstrated.

The presence of fungal endophytes within healthy tissue of vegetables was first described in 1904 (Tan and Zou,2001). Work performed by Samish and Etinger-Tulczynsha (1962) suggested that bacterial endophytes also existed within plants although this was disputed for many years (Lund, 1992). However, it has only recently been accepted that bacteria can indeed reside in the internal structures of undamaged plants (Rosenblueth and Martinez-Romero, 2006; Schulz and Boyle, 2005).

The endophytic bacterial population of plants is known to be diverse, comprising both Gram positive and Gram negative cells (Guo *et al.*, 2008; Torres *et al.*, 2008; Tyler and Triplett, 2008). For example, nitrogen-fixing bacteria from the genus *Azospirillum*, *Herbaspirillum*, *Acetobacter*, *Azoarcus*, and *Burkholderia* spp. are frequently encountered endophytes in nonlegume plants (Baldani *et al.*, 1997). Non-nitrogen-fixing endophytes include species of *Bacillus*, *Pseudomonas*, *Corynebacterium*, *Micrococcus*, *Erwinia*, *Streptomyces*, *Rhodococcus*, *Microlunatus*, and *Luteococcus* (James and Olivares, 1998). Of relevance to the current review, human pathogens have also been detected in surveys of endophytic populations of plants. For example, *Salmonella*, *Staphylococcus*, *Mycobacterium*, *Klebsiella*, and *Burkholderia* have been recovered from endophytic population of plants, thereby supporting the hypothesis that human pathogens can internalize within healthy plant tissue (Rosenblueth and Martinez-Romero, 2006).

However, experimentally demonstrating the internalization of human pathogens has produced conflicting results primarily because of the difficulties in proving that infiltration has actually occurred (Table 4.6).

TABLE 4.6 Internalization of pathogens within growing plants

Comments	Method used to assess internalization	Researchers
Internalization of poliovirus into growing tomato plants but only at high $>10^4$ tita levels	Sand layer between inoculated soil and phyllosphere	Oron *et al.* (1995)
E. coli O157:H7 in radish sprouts	Surface sterilization using 0.2% HgCl	Itoh *et al.* (1998)
Internalization of *Salmonella* into tomatoes by inoculating stems or blossom of plants	Immersion in 70% ethanol for 2 min	Guo *et al.* (2001)
Colonization of gfp labeled *E. coli* O157:H7 at cut edges of leafy greens	No surface sterilization Confocal microscopy to detect gfp label	Takeuchi and Frank (2000)
Internalization of *E. coli* O157:H7 within lettuce seedlings cultivated in soil or hydroponically	No surface sterilization Confocal microscopy to detect gfp label	Wachtel *et al.* (2002)
Internalization of MS2 coliphage into growing cress plants	Inoculated soil was overlaid with agar	Kirkham *et al.* (2002)
Internalization of *E. coli* O157:H7 into lettuce seedlings	No surface sterilization Confocal microscopy to detect gfp label	Solomon *et al.* (2002)
Internalization of nonpathogenic *E. coli* into cabbage seedlings	80% ethanol	Rafferty *et al.* (2003)
Internalization of *E. coli* O157:H7 and *Salmonella* into growing *Arabidopsis* plants when pathogens were introduced into soil	No surface sterilization Confocal microscopy to detect internalized populations	Cooley *et al.* (2003)
Internalization of *E. coli* in spinach plants cultivated in soil or hydroponically	20,000 ppm sodium hypochlorite	Warriner *et al.* (2003a)

Internalization of *E. coli* and *Salmonella* in sprouting mung beans	20,000 ppm sodium hypochlorite	Warriner *et al.* (2003b)
No internalization of *E. coli* O157:H7 in spinach plants when subjected to physical or biological damage	2000 ppm sodium hypochlorite	Hora *et al.* (2005)
Internalization of *E. coli* O157:H7, *Salmonella* and *L. monocytogenes* in growing plants	20,000 ppm sodium hypochlorite	Jablasone *et al.* (2005)
No internalization of *Salmonella* in tomato fruit when inoculum was applied to the roots of growing plants	No surface sterilization Screening for *Salmonella* in ripened fruit	Jablasone *et al.* (2004)
Aggregation of *Salmonella* in stomata and cut cuticle cracks	No surface sterilization	Duffy *et al.* (2005)
Enhancement of internalization by *E. coli* O157:H7 in *Arabidopsis* if coinoculated with *Wausteria paucula*	No surface sterilization	Cooley *et al.* (2006)
Internalization of *Salmonella* in parsley leaves	No surface sterilization	Lapidot and Yaron (2007)
Internalization of *Salmonella* but not *L. monocytogenes* when introduced onto the roots of 4-week-old barley plants	1% chloramine T	Kutter *et al.* (2006)
Internalization of *E. coli* O157:H7 into mature lettuce plants when introduced onto the roots of plants	No surface sterilization. Perforated polypropylene sheet used to physically separate the inoculation site (roots) from the aerial leaves	Bernstein *et al.* (2007)

(*continued*)

TABLE 4.6 (*continued*)

Comments	Method used to assess internalization	Researchers
Internalization of *E. coli* O157:H7 and *Salmonella* in hydroponically cultivated lettuce	1% silver nitrate	Franz *et al.* (2007)
Internalization of different *Salmonella* serovars into tomatoes when introduced onto the plant blossom	2000 ppm calcium hypochlorite	Shi *et al.* (2007)
No internalization of *E. coli* O157:H7 via roots when applied to soil	Surface sterilization using 80% ethanol followed by 0.1% $HgCl_2$	Zheng *et al.* (2008)
No internalization of *E. coli* O157:H7 in leaves when inoculated into the phyllosphere of growing lettuce plants	Surface sterilization using 80% ethanol followed by 0.1% $HgCl_2$	Zheng *et al.* (2008)
No internalization of *E. coli* O157:H7 or *Salmonella* when introduced into the water for irrigating 3–33-day posttransplanted lettuce plants	Surface sterilization using 80% ethanol followed by 0.1% $HgCl_2$	Erickson *et al.* (2008)
Internalization of *E. coli* O157:H7 overexpressing curli into lettuce leaves when applied as a surface inoculum	70% ethanol and visualization using bioluminescent marker	Tanner *et al.* (2008)

Attempting to demonstrate internalization of human pathogens within growing plant tissues is problematic. The traditional approach is to surface sterilize plant material with sanitizers (e.g., sodium hypochlorite, peracetic acid, ethanol) and recover the subsequent bacteria. However, spores (fungal and bacterial) and biofilms are resistant to sanitizers leading to false positive results (Reissinger *et al.*, 2001). Penetration of sanitizer into the internal tissue of plants is a further problem and can potentially lead to an underestimation of endophyte numbers. Therefore, to overcome such limitations, several researchers have taken the approach of carefully inoculating the roots of plants without contacting the leaves which are subsequently screened for the presence of the target pathogen (Franz *et al.*, 2007). The addition of a physical layer (e.g., sand) between the roots and phyllosphere is a further approach to prevent contamination being introduced on, as opposed to within, leaves (Franz *et al.*, 2007). However, the potential for contamination being introduced onto the external surface of leaves exists thereby reducing the confidence that pathogens detected are truly internalized.

An alternative to culturing techniques is the application of cell labeling exploiting green fluorescent protein (gfp) in combination with laser confocal microscopy (Table 4.6). Gfp is a protein originally isolated from the jellyfish *Aequorea victoria*. The key benefit of gfp is the ability to fluoresce under UV light in the absence of an energy source or other cellular cofactors, thereby enabling *in situ* visualization with minimum disruption to cell physiology. The gene encoding for gfp can be readily inserted and expressed in bacterial cells using plasmid vectors. However, for the plasmid to be retained and replicated within the host cell, selective pressure (typically using an antibiotic) needs to be applied. Therefore, when studying plant:microbial interactions over extended periods, selective agents cannot be used and hence the gfp phenotype can be readily lost. A further limitation to gfp labeling is the need for a high cell density in order to visualize bacteria using confocal microscopy. Clearly, if the tagged bacteria are present in low numbers then locating cells within plant tissue is unlikely.

A further method to visualize the presence of internalized bacteria is through the use of glucuronidase (GUS) activity stain. The GUS stain is based on the cleavage of a chromagenic substrate (e.g., 5-bromo-4-chloro-3-indoyl-β-D-glucuronide; X-GLUC) that can be directly visualized as a blue/green precipitate within plant tissues. Therefore, if the target cell is present the chromagen accumulates and hence has greater sensitivity compared to gfp labels. The GUS technique has been used extensively to study plant:bacteria interactions based on *gus* gene insertion into the target bacterium (Wilson *et al.*, 1995). GUS activity is not present in plants or a wide range of bacteria (Wilson *et al.*, 1995), although it is expressed in the majority (>96%) of known generic *E. coli* strains (Liang *et al.*, 2005).

Warriner *et al.* (2003b,c) have used GUS *in situ* staining to demonstrate the internalization of generic *E. coli* in spinach and bean sprouts.

D. Genetic and physiological factors

The finding of a diverse population of endophytes within plants may be unexpected given that the endogenous plant defenses function to guard against microbial invasion. The inducible defenses are commonly activated in response to phytopathogens to contain the site of infection and prime the plant against further microbial attack. At the site of infection the hypersensitive response (HR) is induced, which releases an oxidative burst to cause localized necrosis. At the same time, the plant hormones (e.g., salicylic acid) are circulated to other parts of the plant to activate the systemic acquired resistance (SAR) which primes the defenses. It is thought that endophytic bacteria enhance the resistant of plants to phytopathogens by inducing SAR. The ingress of opportunistic saprophytes into the plant activates the localized induced resistance (LIR) (Newman *et al.*, 2001) which releases antimicrobials within the localized area to suppress saprophytic activity without leading to necrosis (Esposito *et al.*, 2008). Hence, microbes that become established as endophytes do not induce HA and LIR within plants. One strategy developed by phytopathogens and endophytes is to lose flagella or shield Lipopolysaccharide (LPS) which prevent detection, hence activation by plant defenses (Gozzo, 2003; Liu *et al.*, 2007).

Enteric bacteria have been found to differ with respect to the ability to become integrated into the endophytic microflora of alfalfa roots (Dong *et al.*, 2001). A strain isolated from maize, *Klebsiella pneumoniae* 342, colonizes the interior of several host plants in higher numbers compared to *S*. Typhimurium and *E. coli* K12 (Dong *et al.*, 2003). However, a *Salmonella* mutant lacking flagella and Type III secretion system could readily colonize the roots of alfalfa and become integrated into the endophytic microflora. However, restoration of either phenotype reduced the ability of *Salmonella* to colonize roots (Dong *et al.*, 2003). It was thought that the lack of flagella and Type III secretion system prevented the activation of the salicylic acid response or independent response which in turn failed to induce PR1 promoter, and hence release of antimicrobials (Iniguez *et al.*, 2005). The question of if enteric pathogens found naturally in the environment shed their outer surface structures to enhance internalization into plants remains unclear. Nevertheless, it is possible that the ability to prevent activation of plant defenses may explain the interstrain variability that exists with respect to pathogen interactions with plants.

Although plant and animal pathogens infect different hosts, there are several similarities in the strategies employed (Buckhout and Thimm, 2003). For example, Type III secretion systems can be found in both plant and animal pathogens. The Type III secretion system is

essentially a microtube by which the invading bacterium attaches to the surface of the host cell. Chemicals and proteins are delivered through the Tir III protein to sequester defense mechanisms and reprogram host cell activity. Of course, this does not imply that a plant pathogen would cause disease in animals. Indeed, to date only *Ps. auruginosa* PA14 is known to cause disease in both animals and plants (Plotnikova *et al.*, 2000). Nevertheless, it is conceivable that traits in phytobacteria associated with plant interactions could be present in enteric pathogens such as *E. coli* O157:H7. It is known that broad host range bacteriophage that infects *Pseudomonas* and *E. coli* O157:H7 can transfer genetic traits between these two genera (Hendrix, 1999; Muniesa *et al.*, 2003). An example of phytobacteria sharing genes with enteric pathogens has been found in *Ps. syringae* pv. *maculicola*. The phytopathogen posses a β-lactamase which protects the bacterium against preformed defenses in *Arabidopsis*. The gene encoding for the enzyme, donated as *sax* (Survival on *Arabidopsis* eXtract), has been identified in a range of *Ps. syringae* pathovars but absent from non-phytopathogenic strains. From comparative homology studies, the gene shows a high level of similarity to an uncharacterized gene in *E. coli* O157:H7 (Crooks and Lamb, 2001). Whether the expression of *sax* within *E. coli* O157:H7 enhances persistence within plants has yet to be established but does point to an adaptive response of human pathogens to survive outside of the host environment. However, this view remains contentious with many researchers in the field, suggesting that the interaction of human pathogens with plants is a passive process being comparable to any oppertunistic saprophyte (Doyle and Erickson, 2008). Yet, as previously indicated, there is accumulating evidence to support the hypothesis that human pathogens have evolved specialized mechanisms for becoming established and persisting on plants.

It has been observed that pathogens such as *Salmonella* and *E. coli* O157:H7 preferentially attach to cut surfaces and natural openings such as stomata, whereas common epiphytes such as *Ps. fluoresecens* colonize the intact plant tissue (Li *et al.*, 2008; Melotto *et al.*, 2006; Seo and Frank, 1999; Takeuchi and Frank, 2001). It is known that attachment of human pathogens is an active process which requires the bacterium to be in a viable state although the actual internalization process can be passive (Solomon and Matthews, 2006).

It is thought that cell surface components such as cellulose, flagella, pilli, and Type III secretion systems all play a role in cell attachment (Barak *et al.*, 2005; Zogaj *et al.*, 2001). Several genes and mechanisms have been identified as being involved in attachment of human pathogens to plants. These mechanisms include curli, fimbriae, adhesins, and capsule production (Barak *et al.*, 2005, 2007).

From microarray studies it has been demonstrated that virulence genes are upregulated in *S.* Typhimurium during colonization in response to lettuce root exudates (Klerks *et al.*, 2007) which aid attachment to the plant tissue. It is also observed that genes involved in sugar–phosphate metabolism are also upregulated which is thought to attract the enteric pathogen to cut edges of damaged leaves (Klerks *et al.*, 2007). The presence of structures on the plant cell walls has also been proposed. This hypothesis is supported by the fact that attachment of pathogens such as *E. coli* O157:H7 and *Salmonella* is plant specific. For example, attachment of pathogens is more frequently observed with *Brassicaceae* compared to lettuce, carrots, or tomatoes (Barak and Liang, 2008). Collectively, studies to date support the view that human pathogens have adapted to colonize plants as a means of persisting between animal or human hosts.

VI. INTERVENTIONS TO ENHANCE THE SAFETY OF FRESH PRODUCE

Postharvest washing of vegetables remains the key intervention to remove field acquired contamination. There have been numerous papers and reviews on the relative performance of different sanitizers including hypochlorite, chlorine dioxide, and peroxyacetic acid (Allwood *et al.*, 2004; Gonzalez *et al.*, 2004; Ibarra-Sanchez *et al.*, 2004; Koseki and Isobe, 2006; Rodgers *et al.*, 2004; Romanova *et al.*, 2002).

From reviewing the literature, typical log reductions achieved for *Salmonella* on lettuce cover a wide range: peroxyacetic acid 1.7 (Hellstrom *et al.*, 2006), acidified sodium chlorite 3.1 (Inatsu *et al.*, 2005), chlorine dioxide 1.53 (Inatsu *et al.*, 2005), ozone 5.6 (Rodgers *et al.*, 2004), and electrolyzed water 1.0 (Koseki and Isobe, 2006; Koseki *et al.*, 2003). However, on natural contaminated produce the log count reductions achieved are typically 1–2 log cfu/g regardless of the sanitizer applied (Doyle and Erickson, 2008). The limitation of postharvest washes can be attributed internalized populations or those within biofilms on the surface of produce (Gonzalez *et al.*, 2004; Koseki *et al.*, 2003). The heavy organic loading of wash water can also readily neutralize sanitizers such as hypochlorite. This can be addressed to a degree by operating at a set oxidation–reduction potential (ORP). Here, the amount of chlorine introduced into the water is increased in the presence of organic matter to maintain the chlorous acid levels within the wash. However, even with ORP-controlled systems the LCR is not significantly improved compared to when chlorine washes alone are applied (Guentzel *et al.*, 2008).

Postharvest washing does not only provide a low level of confidence of decontaminating produce but is also thought to result in

cross-contamination between fresh produce batches. It is known that contaminated flume water is a potential source of contamination of *Salmonella* for fruits such as tomatoes (Zhuang *et al.*, 1995). Luo (2007) reported that lettuce washed in recycled chlorinated water with high total solids content spoiled more rapidly compared to samples washed in fresh chlorinated water. More direct evidence for cross-contamination via wash water was reported by Ilic *et al.* (2008). The researchers evaluated the effect of commercial wash process on the coliform and *E. coli* counts associated with spinach. The finding of the study was that the proportion of samples positive for coliforms increased from 53% to 79% following washing (Ilic *et al.*, 2008). It is possible that the increase in coliform prevalence was caused by the uptake of wash water caused by a temperature differential effect. It is known that warm produce placed into cold water results an influx of wash water (hence contamination) into the inner vascular system of leafy greens and fruit (Bolton *et al.*, 2002; Fukumoto *et al.*, 2002; Ibarra-Sanchez *et al.*, 2004). To overcome the problem of infiltration, it is recommended to use warm water for washing produce (FDA, 1998). However, this has the adverse effect of warming the produce, thereby accelerating plant autolysis and growth of spoilage microbes or even human pathogens.

Surface pasteurization of produce using steam, hot water, or chlorine dioxide gas was shown to enhance the reduction of microbial loads on hard surface produce (Stringer *et al.*, 2007). However, delicate produce such as leafy vegetables can be damaged by the steam process (Allwood *et al.*, 2004; McWatters *et al.*, 2002; Sy *et al.*, 2005). A study conducted by Sapers and Sites (2003) showed that cantaloupe treated with hot (80 °C) 5% hydrogen peroxide for 3 min reduced *E. coli* and *Salmonella* populations with no signs of tissue damage after 26 days of storage at 4 °C. However, some disadvantages with heat treatments for produce occur. Heat treatment can reduce microbial loads on produce but has little effect if contaminated after heating. A study by Conway *et al.* (2005) showed that injured apples had larger microbial loads of pathogens when heat treated. This was most likely due to the damage of enzymes that are the plant's defense system against invasion of microorganisms. Overall, the quality of commodities such as fresh cut lettuce diminishes during extended storage after heat treatment of more than 3 min.

Irradiation of produce has been shown to be effective in reducing microbial contamination where the maximum dosage level is 1.0 kGy for fruits and vegetables (Bari *et al.*, 2005; Gomes *et al.*, 2008; Niemira, 2007). An irradiation dose of 1.0 kGy treatment decreased mesophilic bacteria in Mexican salads (Erickson, 2008). However, irradiation can cause changes in pectin structure leading to texture loss and hence shelf life. Also, viruses seem to be relatively resistant to irradiation treatment and relative to vegetative cells, suggesting that doses delivered to inactive

pathogens (e.g., *Salmonella*) would be insufficient to kill NLV (Gomila *et al.*, 2008).

High hydrostatic pressure (HHP) processes have been used mainly for sauces or seafood and proven effective at reducing microbial populations without adverse effects on product quality (Considine *et al.*, 2008; Brinez *et al.*, 2006). HHP treatment causes bacterial inactivation by damaging the cell membrane, which affects membrane permeability and intracellular enzyme inactivation and possibly ruptures the plant cell wall (Kniel *et al.*, 2007). Although HHP have proven to be one of the most effect decontamination intervention strategies, there is the potential of causing disruption to plant tissue leading to accelerated spoilage (Basak and Ramaswamy, 1998; Butz *et al.*, 2002; Préstamo and Arroyo, 1998). Pressure-induced damage to plant tissue results from the stress and strains imposed on the cell walls which subsequently results in loss of texture (Fuchigami *et al.*, 1995; Hartmann and Delgado, 2004; Kidmose and Martens, 1999). However, it has also been reported that HHP treatment can enhance the texture of vegetables through de-esterifying pectin via the activity of pectin methyltransferase (PMT) which facilitates calcium binding to stabilize plant cell walls (Fennema, 1996; Sila *et al.*, 2004).

UV light has been considered as an alternative to sanitizer-based systems for decontaminating fresh produce (Bialka and Demirci, 2008). Unlike chemical sanitizers, UV does not leave residues and the product does not need to be dried thereby providing energy savings (Bialka and Demirci, 2008). UV light can be divided into three classes: UV-A (400–320 nm), UV-B (320–280 nm), and UV-C (<280 nm) where the most effective range for produce decontamination is within 200–280 nm. UV-C treatments are effective at decreasing psychrotrophic bacteria, coliforms, and yeasts and molds. However, bacterial spores and stationary phase cells are much more resistant to UV-C light than vegetative and exponential phase cells. A study by Yaun *et al.* (2004) reported that a greater than 9 mW/cm^2 dose of UV-C light reduced microbial populations by 2 log in lettuce and tomatoes and 3 log in apples.

There are some disadvantages associated with UV-C-treated produce, which include possible negative effects on quality at high doses and poor penetration. Also, UV-C treatment was more effective for produce with smoother surfaces and did not significantly reduce viruses like norovirus and FCV (Fino and Kniel, 2008).

UV light has been combined with hydrogen peroxide to develop a produce decontamination that can be used to inactivate pathogens on the surface and internalized contamination (Hadjok *et al.*, 2008; Xie *et al.*, 2008). The underlying principle of the approach is to generate highly reactive, antimicrobial, radicals from the UV degradation of hydrogen peroxide in a process termed advanced oxidative process (AOP) (Rodgers *et al.*, 2004; Suty *et al.*, 2004). By using a 50 °C H_2O_2 (1.5%) simultaneously

applied with UV-C, it was possible to achieve >5 log reductions of human pathogens and viruses (MS2 phage surrogate) on leafy greens (Hadjok *et al.*, 2008; Xie *et al.*, 2008).

A. Biocontrol of human pathogens

Control of human pathogens at the primary production level is problematic due to its open nature. It has been proposed that decontamination of irrigation water through ozonation or chlorination is an option although the cost and effect on the plant microecology are obvious limitations (Ajwa *et al.*, 2002; Rojas-Valencia *et al.*, 2004). A more practical approach is to use biocontrol strategies whereby antagonistic microbes are introduced into the rhizosphere to reduce or inhibit pathogens. To date the majority of biocontrol strategies have been focused on controlling phytopathogens (Vassilev *et al.*, 2006). The use of biocontrol strategies to control human pathogens is an emerging area but has yet to be studied in detail. It has been previously reported that *Enterobacter* is antagonistic against *Salmonella* introduced onto *Arabidopsis* and lettuce (Cooley *et al.*, 2003, 2006). Although the mechanisms are unknown, it is thought that *Enterobacter* can compete more effectively for nutrients utilized by *Salmonella* to support growth and persistence. Fett *et al.* (2006) introduced a *Pseudomonas fluorescens* strain (isolated from the rhizosphere of wheat) into the soak water of *Salmonella* inoculated alfalfa seeds. When the seeds were sprouted that sprouts with *Ps. fluorescens* had 5 log lower *Salmonella* levels compared to controls (Fett *et al.*, 2006). Matos and Garland (2005) introduced a cocktail of bacteria (unknown composition) obtained from germinated sprouts to alfalfa seeds inoculated with *Salmonella*. The *Salmonella* counts on sprouts derived from seed treated with biocontrol agent were 5.7 logs lower compared to controls. However, because the bacterial cocktail was directly obtained from sprouts, attempting to reproduce the composition on a commercial scale could be problematic. The application of bacteriophage has been evaluated for controlling human pathogens although with various degrees of success (Guenther *et al.*, 2008; Hudson *et al.*, 2005). Bacteriophages are viruses that infect and replicate within bacterial hosts. Advantages of phages are that they are specific, self-perpetuating, and self-limiting. Although widely used in Eastern Europe, bacteriophages have only recently being approved for food applications. In 2006, the Food and Drug Administration (FDA) approved a *L. monocytogenes*-specific phage preparation (LMP-102) for use as an antimicrobial agent against *L. monocytogenes* contamination of ready-to-eat foods (Guenther *et al.*, 2008; Lang, 2006; Leverentz *et al.*, 2001). In relation to fresh produce, Abuladze *et al.* (2008) evaluated a cocktail (designated ECP-100) of phages to control *E. coli* O157:H7 on a variety of vegetable types. The researchers reported a 94–99% reduction of *E. coli* O157:H7

introduced onto tomatoes and complete inactivation with spinach inoculated with 14,000 cfu of the pathogen. However, it is possible that infection and phage replication occurred during cultivation of survivors given that phage numbers on the fresh produce samples remained the same (Abuladze *et al.*, 2008). Leverentz *et al.* (2004) reported a 2–3 log reduction of *Salmonella* on melon although complete elimination of the enteric pathogen was not observed.

VII. CONCLUSIONS AND FUTURE RESEARCH

Despite the increased awareness of food safety issues surrounding fresh produce, the number and frequency of foodborne illness outbreaks continue to rise. It is evident that the centralization of production coupled with the growth in the bagged salad market are significant factors to explain the incidence of foodborne illness linked to fresh cut produce. However, environmental factors also play a significant role especially in terms of manure management in disseminating human pathogens to water courses and/or soil.

The adaption of human pathogen strains to grow and persist on plants remains a relatively unexplored area. In many aspects, the adaption of human pathogens to plants would make sense given that the microbes need to survive in the environment between infecting hosts.

The finding that human pathogens can contaminate growing crops in the field and persist through to consumption is of concern and interventions above simple postharvest washing have to be considered. One strategy of interest is through implementing biocontrol methods that can control human pathogens at the primary production level.

With regards to postharvest control, it can be envisaged that more effective decontamination treatments will be adopted such as AOP or more likely irradiation. However, regardless of technological advances there will always be a role of GAP (Good Agricultural Practice) and GMP (Good Manufacturing Practice) for enhancing the microbiological safety of fresh produce.

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