

REVIEW ARTICLE

Microbial contamination of fruit and vegetables and the behaviour of enteropathogens in the phyllosphere: a review

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Summary

Consumption of fruit and vegetable products is commonly viewed as a potential risk factor for infection with enteropathogens such as *Salmonella* and *Escherichia coli* O157, with recent outbreaks linked to lettuce, spinach and tomatoes. Routes of contamination are varied and include application of organic wastes to agricultural land as fertilizer, contamination of waters used for irrigation with faecal material, direct contamination by livestock, wild animals and birds and postharvest issues such as worker hygiene. The ability of pathogens to survive in the field environment has been well studied, leading to the implementation of guidelines such as the Safe Sludge Matrix, which aim to limit the likelihood of viable pathogens remaining at point-of-sale. The behaviour of enteropathogens in the phyllosphere is a growing field of research, and it is suggested that inclusion in phyllosphere biofilms or internalization within the plant augments the survival. Improved knowledge of plant–microbe interactions and the interaction between epiphytic and immigrant micro-organisms on the leaf surface will lead to novel methods to limit enteropathogen survival in the phyllosphere.

Introduction

It is now commonly accepted that fruit and vegetable consumption is a risk factor for infection with enteric pathogens. Recent examples of outbreaks related to fresh produce include cases of *Escherichia coli* O157:H7 (spinach, lettuce), *Salmonella* Typhimurium and *S. Newport* (tomatoes, lettuce), *S. Thompson* (rocket) and hepatitis A (spring onion). Table 1 gives a comprehensive list of recent outbreaks.

Increased consumption of fresh fruit and vegetables

Consumption of fresh produce has increased (Anon, 2007) mainly because of heightened awareness of the benefits of a healthy diet and the impact of the 'Five a Day' and 'Nine a Day' governmental campaigns in the UK and USA. This has led to consumer demand for improved choice, such as minimally processed, prepacked, ready-to-

eat fruit and vegetables (Everis 2004) and availability of out-of-season produce.

Prepared produce, such as bagged salads, may provide the conditions for the proliferation and survival of human pathogens. Cut surfaces exude nutrients, which can be utilized for growth and the pathogens *E. coli* O157:H7 and *Listeria monocytogenes* have been shown to attach to the cut surfaces of lettuce leaves and penetrate the internal tissue, indicating protection from chemical sanitizers (Takeuchi and Frank 2000). However, surveys of bagged salads often recover low numbers of enteropathogens (Szabo *et al.* 2000; Francis *et al.* 1999; Lin *et al.* 1996). Adak *et al.* (2005) calculated the disease risks of food groups and reported a low risk ratio for salad vegetables, although fresh produce was reported to have a considerable, if occasional, impact on disease incidence in a population.

Another factor that impacts directly on microbial quality is the increase in importation to meet consumer

Table 1 Reported fresh-produce related outbreaks of *Salmonella*, *Escherichia coli* and *Campylobacter*, 1996 onwards and viral gastroenteritis, 1990 onwards

Year	Pathogen	Vehicle	Reference
1997	<i>Salmonella</i> Enteritidis	Cauliflower	Anon (2005a)
1997	<i>S.</i> Enteritidis PT4	Pepper	Anon (2005a)
1997	<i>S.</i> Meleagridis	Sprouted seeds (alfalfa)	Taormina <i>et al.</i> (1999)
1997	<i>S.</i> Infantis/ <i>S.</i> Anatum	Sprouted seeds (alfalfa and mung bean)	Taormina <i>et al.</i> (1999)
1997	<i>S.</i> Saphra	Cantaloupe	Sivapalasingham <i>et al.</i> (2004)
1997–8	<i>S.</i> Senftenberg	Sprouted seeds (clover and alfalfa)	Taormina <i>et al.</i> (1999)
1998	<i>S.</i> Havana/ <i>S.</i> Cubana/ <i>S.</i> Tennessee	Sprouted seeds (alfalfa)	Taormina <i>et al.</i> (1999)
1998	<i>S.</i> Oranienburg	Cantaloupe	Anon (2001a,b)
1998–99	<i>S.</i> Baildon	Tomatoes	Anon (2001a,b)
1999	<i>S.</i> Muenchen	Unpasteurized orange juice	Anon (2001a,b)
1999	<i>S.</i> Thompson	Cilantro	Sivapalasingham <i>et al.</i> (2004)
1999	<i>S.</i> paratyphi B var. <i>java</i>	Sprouted seed products	Stratton <i>et al.</i> (2001)
2000	<i>S.</i> Enteritidis	Unpasteurized citrus juice	Anon (2001a,b)
2000	<i>S.</i> Poona	Cantaloupe	CDC (2002a)
2000	<i>S.</i> Typhimurium	Lettuce	Horby <i>et al.</i> (2003)
2000	<i>S.</i> Typhimurium DT104	Lettuce	Anon (2005a)
2000	<i>S.</i> Typhimurium DT204B	Lettuce	Anon (2005a)
2000	<i>S.</i> Enteritidis 11b	Mung bean sprouts	Harb <i>et al.</i> (2003)
2001	<i>S.</i> Kottbus	Sprouted seeds (alfalfa)	CDC (2002b)
2001	<i>S.</i> Newport	Mixed bagged salad	Anon (2005a)
2001	<i>S.</i> Virchow	Salad items	Anon (2005a)
2001	<i>S.</i> Poona	Cantaloupe	CDC (2002a)
2001	<i>S.</i> Enteritidis	Mung bean sprouts	Honish and Nguyen (2001)
2002	<i>S.</i> Javiana	Tomatoes	CDC (2002c)
2002	<i>S.</i> Poona	Cantaloupe	CDC (2002a)
2003–4	<i>S.</i> Enteritidis	Almonds	CDC (2004)
2004	<i>S.</i> Newport	Lettuce	Gillespie (2004)
2004	<i>S.</i> Thompson	Rocket salad	Nygaard <i>et al.</i> (2004)
2004	<i>S.</i> Braenderup	Tomatoes	CDC (2005)
2004	<i>S.</i> Javiana	Tomatoes	Anon (2005a)
2005	<i>S.</i> Typhimurium DT104	Spanish lettuce	Takkinen <i>et al.</i> 2005
2005	<i>S.</i> Typhimurium DT104	Lettuce	Anon (2005a)
2005	<i>S.</i> Enteritidis	Bean sprouts	Anon (2005b)
2006	<i>S.</i> Newport	Tomatoes	Anon (2007)
2006	<i>S.</i> Typhimurium	Tomatoes	CDC (2006a)
2007	<i>S.</i> Senftenberg	Basil	Pezzoli <i>et al.</i> (2007)
1997	<i>E. coli</i> O157:H7	Sprouted seeds (alfalfa)	Sivapalasingham <i>et al.</i> 2004
1997	<i>E. coli</i> O157:H7	Salad	Anon (2005a)
1998	<i>E. coli</i> O157:H7	Salad	Anon (2001a,b)
1998	<i>E. coli</i> O157:H7	Fruit salad	Anon (2001a,b)
1998	<i>E. coli</i> O157:H7	Coleslaw	Anon (2001a,b)
1998	<i>E. coli</i> O157:H7	Sprouted seeds (clover/alfalfa)	Taormina <i>et al.</i> 1999
1998	<i>E. coli</i> O157:H7	Unpasteurized apple juice	Anon (2001a,b)
1998	<i>E. coli</i> O157:H7	Parsley	Sivapalasingham <i>et al.</i> 2004
1999	<i>E. coli</i> O157:H7	Coriander (cilantro)	Campbell <i>et al.</i> 2001
1999	<i>E. coli</i> O157:H7	Unpasteurized apple juice	Anon (2001a,b)
2003	<i>E. coli</i> O157:H7	Cucumber	Duffell <i>et al.</i> (2003)
2003	<i>E. coli</i> O157:H7	Lettuce	Anon (2005a)
2005	<i>E. coli</i> O157:H7	Lettuce	Söderström <i>et al.</i> (2005)
2006	<i>E. coli</i> O157:H7	Spinach	CDC (2006b)
2006	<i>E. coli</i> O157:H7	Lettuce	CDC (2006c)
1996	<i>Campylobacter jejuni</i>	Salad items	Mandrell and Brandl (2004)
1996	<i>C. jejuni</i>	Lettuce*	CDC (1998)
1996	<i>C. jejuni</i>	Lettuce	Anon (2005a)
1997	<i>C. jejuni</i>	Sweet potatoes†	Harriman 1998

Table 1 *Continued*

Year	Pathogen	Vehicle	Reference
1997	<i>C. jejuni</i>	Cucumber‡	Kirk <i>et al.</i> 1997
2000	<i>C. jejuni</i>	Lettuce	Anon (2005a)
2001	<i>C. jejuni</i>	Orange juice	Anon (2005a)
1990	Norovirus	Fresh cut fruit	Herwaldt <i>et al.</i> (1994)
1990	Hepatitis A	Iceberg lettuce	Seymour and Appleton (2001)
1992	Norovirus	Lettuce/tomato	Anon (2005a)
1992	Norovirus	Melon	Anon (2005a)
1992	Hepatitis A	Strawberries	Seymour and Appleton (2001)
1994	Hepatitis A	Tomatoes§	Anon (2001a)
1994	Norovirus	Salad	Anon (2005a)
1994	Norovirus	Carrot	Anon (2005a)
1994	Norovirus	Salad	Anon (2005a)
1995	Norovirus	Salad	Anon (2005a)
1995	Norovirus	Salad	Anon (2005a)
1995	Hepatitis A	Diced tomatoes	Seymour and Appleton (2001)
1997	Hepatitis A	Frozen strawberries§	Anon (2001a)
1997	Unknown virus	Orange juice	Anon (2005a)
1998	Norovirus	Fruit salad	Anon (2005a)
1998	Norovirus	Fruit salad	Anon (2005a)
1998	Hepatitis A	Salad items	Seymour and Appleton (2001)
1999	Norovirus	Salad items	Anon (2005a)
1999	Norovirus	Salad items	Anon (2005a)
2001	Norovirus	Raspberries	Le Guyader <i>et al.</i> (2004)
2002	Norovirus	Salad items	Anon (2005a)
2002	Norovirus	Fruit salad	Anon (2005a)
2004	Hepatitis A	Spring onion§	Josefson (2003)
2005	Norovirus	Raspberries	Korsager <i>et al.</i> (2005)
2005	Norovirus	Raspberries	Cotterelle <i>et al.</i> (2005)
2006	Norovirus	Raspberries	Hjertqvist <i>et al.</i> (2006)

*Cross-contamination from chicken juices.

†Cross-contamination either with meat juices or kale garnish.

‡Contamination during storage or processing.

§Thought to be due to contact with an infected food handler.

expectations for a wide choice of exotic fruit and vegetables year-round. As standards of hygiene at harvest, during storage, and of irrigation water can vary widely in different countries, the potential for contamination of produce may be increased and consumers may be exposed to high numbers and/or different strains of pathogens. Imported produce from Mexico has been shown to be of an equivalent microbial quality to domestic samples in the USA (Johnston *et al.* 2006), but a number of outbreaks have been linked to imported produce, for example lettuce imported into the UK from Spain during 2005 (Takkinen *et al.* 2005).

The increase in outbreaks related to fresh produce can, in part, be attributed to changing consumer habits. A Food Standards Agency survey completed in the UK (Anon 2007) showed that 63% of people used large supermarkets for the majority of food shopping, whilst only 2% used specialist local stores. If contaminated items

enter the large-scale production facilities relied upon by supermarkets, affected final products are likely to reach an increased number of consumers. Outbreaks are more likely to be identified than sporadic cases. Indeed, the number of sporadic cases linked to the consumption of contaminated fresh fruit and vegetables is unknown. Improved surveillance programmes have resulted in outbreaks being more readily identified, with fresh produce now increasingly likely to be considered a vehicle of infection and therefore included in any investigation.

During April 2007, two major recalls of bagged lettuce occurred in the UK after *Salmonella* contamination was detected. Product recalls, such as these, damage consumer confidence and are economically damaging for the industry, which makes the high investment in microbiological surveillance an economic necessity.

An improved understanding of the behaviour of enteropathogenic bacteria on salad vegetables should help

prevent both the initial contamination events and microbial persistence up to the point-of-sale (POS).

Significant pathogens

Bacteria

Salmonella

Salmonella spp. are the most commonly identified aetiological agent associated with fresh produce-related infection, isolated in 48% of cases between 1973 and 1997 in the USA (Sivapasingham *et al.* 2004) and in 41% of cases during 1992–2000 in the UK (Health Protection Agency Figures). During 2006, two major tomato-related *Salmonella* outbreaks in the USA accounted for 23.2% of reported *Salmonella* cases (CDC 2007).

A range of fresh fruit and vegetable products have been implicated in *Salmonella* infection, most commonly lettuce, sprouted seeds, melon and tomatoes. *Salmonella* spp. are often isolated from produce sampled in routine surveys, including lettuce, cauliflower, sprouts, mustard cress, endive and spinach (Thunberg *et al.* 2002) and mushrooms (Doran *et al.* 2005).

Escherichia coli O157:H7

Escherichia coli O157:H7 can be isolated from the faeces of livestock; therefore, its presence in animal manures and slurries is inevitable (Kudva *et al.* 1998). In addition to farm animals, *E. coli* O157:H7 may be present in the faeces of wild birds, for example, starlings (Moller Nielsen *et al.* 2004) and gulls (Wallace *et al.* 1997). Leafy vegetables are most commonly linked to *E. coli* infection, but apple juice (cider in the USA) is an interesting vehicle, as the acidity of the product is considered inhibitory to bacterial proliferation. *Escherichia coli* O157:H7 is commonly recovered from the faeces of ruminants; therefore, livestock grazing in orchards may contaminate fallen apples with faeces and, as *E. coli* O157:H7 can proliferate in damaged apple tissue (Stopforth *et al.* 2004), this can result in the contamination of unpasteurized fruit juices/ciders. Janisiewicz *et al.* (1999) demonstrated that fruit flies were an important vector in the contamination of apples with *E. coli* O157:H7, both preharvest and in packing houses.

Listeria monocytogenes

Listeria spp. are ubiquitous in the environment and can be isolated from soil, water, vegetation, the faeces of livestock and vegetation irrigated with contaminated water. The potential for environmental *Listeria* to contaminate fresh produce and lead to enteric infection has long been recognized (Blakeman 1985) and Harvey and Gilmour (1993)

suggested this most probably occurred during processing. The predominant *L. monocytogenes* serotype isolated from salad vegetables has been shown to be serogroup 1 (Harvey and Gilmour 1993; Heisick *et al.* 1989).

Beuchat (1998) reports a number of surveys documenting the presence of *L. monocytogenes* on cucumber, peppers, potato, radish, leafy vegetables, beansprout, broccoli, tomato and cabbage at POS. These surveys show variation in prevalence on different types of produce and between countries. Arumugaswamy *et al.* (1994) isolated *L. monocytogenes* from beansprout (85%), leafy vegetables (22%) and cucumber (80%) in Malaysia. In comparison, only 6.7% of cucumbers sampled in Pakistan yielded *L. monocytogenes* (Beuchat 1998). Two surveys of cabbage in Canada reported a prevalence of 2.2% (Schlech *et al.* 1983) and 6.7% (Odumeru *et al.* 1997) respectively, but a larger study in the US reported only 1.1% positive samples (Heisick *et al.* 1989). A US survey of potatoes reported a higher incidence (27.1%) than a survey conducted in Spain (16.7%) (Beuchat 1998) and two separate studies of radish in the US reported results of 14.4% and 36.8% prevalence. Generally, contamination is higher on root vegetables and Heisick *et al.* (1989) suggest that this is due to increased contact with soil. Variations in sample size, sampling regimes and laboratory protocols will also impact on the likelihood of isolation and therefore prevalence data.

Szabo *et al.* (2000) surveyed 120 bagged lettuce samples in Australia and reported isolation of *L. monocytogenes* in 2.5% samples. Thunberg *et al.* (2002) sampled a range of fresh produce and isolated *L. monocytogenes* only from potatoes (50%) and field cress (18%) purchased at farmers' markets. These varied results are likely to represent differences in production practices between countries, within countries and between outlets. This indicates the huge natural variability in the microbial quality of produce.

Crépet *et al.* (2007) analysed 165 studies and reported that prevalence on salad vegetables is usually under 5%, with lower numbers isolated from leafy salad vegetables than from sprouted seeds and other vegetables (e.g. carrots, cabbage, celery and spinach). This paper also showed that surveys conducted after 2000 reported lower instances of *L. monocytogenes* isolation, suggesting that increased knowledge of the behaviour of the pathogen in the food processing environment and more effective sanitization procedures have led to improved product control.

Listeria monocytogenes will grow at refrigeration temperatures (Wonderling *et al.* 2004) therefore is likely to multiply during storage if present on fresh produce. Beuchat and Brackett (1990) showed that *L. monocytogenes* is capable of growth on lettuce when exposed to processing conditions, although carrot juice seemed inhibitory.

Farber *et al.* (1998) demonstrated that *L. monocytogenes* populations declined on grated carrot by 2-logs over 9 days.

Although *Listeria* contamination of fresh produce and survival up to POS seems likely, outbreaks linked to fresh produce are infrequent and tend to be limited to vulnerable groups. The two documented outbreaks which have occurred, in 1979 and 1981 respectively, were attributed to cabbage (in coleslaw) and salad items (celery, lettuce and tomatoes) served as part of hospital meals (Anon 2001a,b). The high infective dose of *L. monocytogenes*, $\sim 10^6$ cells, variation in susceptibility in the population and long incubation period may also explain the scarcity of recorded fresh produce-related outbreaks (McLauchlin *et al.* 2004).

Campylobacter jejuni

Campylobacter jejuni is the most common cause of gastrointestinal illness worldwide, affecting over 2 million people in the USA and 50 000 throughout England and Wales annually (Evans *et al.* 2003) but cases are usually sporadic. Outbreaks are most commonly linked to poultry or cross-contamination from poultry products (Gillespie *et al.* 2003). As *Campylobacter* cannot grow outside of a warm-blooded host, survival on fresh produce is limited, especially if not protected from UV light (Obiri-Danso *et al.* 2001).

Epidemiological evidence suggests that salad vegetables are the second-highest risk factor for *Campylobacter* infection after poultry (Evans *et al.* 2003), and outbreaks have been linked to lettuce, sweet potatoes, cucumber, melon and strawberries (Brandl *et al.* 2004). Kumar *et al.* (2001) isolated *C. jejuni* from spinach, fenugreek, lettuce, radish, parsley, green onions, potatoes and mushrooms and Park and Sanders (1992) reported *Campylobacter* spp. on 1.6–3.3% vegetables tested, but other extensive studies of raw organic and prepack salad vegetables failed to isolate *Campylobacter* (Evans *et al.* 2003). It is suggested that outbreaks linked to fresh produce may be due to cross-contamination in the kitchen (Evans *et al.* 2003).

Aeromonas

Aeromonas spp. are ubiquitous in water, soil, faeces and on vegetation (McMahon and Wilson 2001). *Aeromonas* has been isolated from a wide range of fresh produce including sprouted seeds, asparagus, broccoli, cauliflower, carrot, celery, cherry tomatoes, courgette, cucumber, lettuce, mushroom, pepper, turnip and watercress (Merino *et al.* 1995). Watercress was found to yield higher numbers of *Aeromonas* than lettuce or escarole (chicory) samples (Saad *et al.* 1995). Thirty-four percent of organic vegetables were found to be contaminated with *Aeromo-*

nas (McMahon and Wilson 2001) compared with 26% of conventionally cultivated vegetables (Neyts *et al.* 2001). Growth of *Aeromonas* will occur on shredded lettuce, chicory (Jacxens *et al.* 1999) and tomatoes (Velazquez *et al.* 1998) but not on carrots or Brussels sprouts (Jacxens *et al.* 1999). Callister and Aggar (1987) found that 48% *Aeromonas* isolates from fresh vegetables were *A. hydrophila*, the species most often linked to disease in humans.

Food isolates of *Aeromonas* have been shown to tolerate low pH and to grow at the refrigeration temperatures relied upon for preservation throughout the fresh produce supply chain (Merino *et al.* 1995).

It has been suggested that *Aeromonas* may be responsible for the many gastrointestinal infections ($\sim 40\%$) for which no aetiological agent is found (McMahon and Wilson 2001). However, results from volunteer studies are inconclusive and investigations of foodborne outbreaks fail to indisputably establish *Aeromonas* as the causative agent (Isonhood and Drake 2002). A link has been suggested between the increased incidence of *Aeromonas* in human stools and on fresh vegetable samples during the summer months (Saad *et al.* 1995). Neyts *et al.* (2001) suggest that *Aeromonas* may only pose a risk to immunocompromised groups.

Viral pathogens

Virus particles, such as those of norovirus and hepatitis A, are excreted by an infected host or released in vomit, therefore are likely to be present in sewage and faecally contaminated water. During the period 1992–99, viruses accounted for a similar number (*c.* 20%) of produce-related infections as *Salmonella* (Seymour and Appleton 2001). Enteric viruses have a low infective dose, and remain active even after exposure to low pH (<3) (Seymour and Appleton 2001) and temperature extremes. Freezing, for example, augments virus persistence (Le Guyader *et al.* 2004). Contaminated produce is therefore likely to remain infectious to POS, regardless of processing conditions, although Bidawid *et al.* (2001) suggest that exposure to plant metabolites such as phenolics may cause loss of activity.

Irrigation with sewage-contaminated water was linked to hepatitis A outbreaks associated with lettuce consumption (Seymour and Appleton 2001) and spring onions (Josefson 2003). Contact with an infected food handler can also contaminate food and as viruses can remain viable on inanimate objects and surfaces, it can be simple for poor hygiene practices to lead to food contamination. A number of outbreaks have been directly traced to infected food preparers or pickers (Seymour and Appleton 2001).

Routes of contamination

Organic fertilizers

Use of organic fertilizers, such as animal manures and slurries (Beuchat 1996; Natvig *et al.* 2002), abattoir wastes (Avery *et al.* 2005) and sewage sludge (Al-Ghazali and Al-Azawi 1990) introduce pathogens directly to the field, and run-off can contaminate irrigation water.

Over 90 million tonnes of animal waste are put to land annually in the UK (Food Standards Agency 2004a, Aviation House, London, UK). There are comprehensive guidelines available to growers that advise on sufficient treatment of wastes and correct timing of application, with the aim of limiting contamination of crops. In the UK, these guidelines are set out in the Safe Sludge Matrix (ADAS) (Anon 2001b) and the Codes of Good Agricultural Practice (Department of the Environment, Food and Rural Affairs). The Safe Sludge Matrix, for example, states that even when enhanced-treated sludge is applied to land, a 10-month harvest interval is necessary and the use of conventionally treated sludge requires a 30-month harvest interval for salad crops. These intervals should be sufficient to ensure the microbial quality of produce at harvest. Similar recommendations are set out in the United States Environment Protection Agency Part 503 Biosolids Rule (Anon 1993) and Canadian Ministry of the Environment guidelines (Anon 1996).

Irrigation water quality

Faecal material, soil and other inputs such as sewage overflow introduce enteropathogens directly to water-courses from which irrigation water may be extracted. In the UK, 71% of irrigation water is obtained from surface waters, which receive treated sewage effluent (Tyrell *et al.* 2006). The potential for contamination via irrigation water is increased in the developing world, as untreated wastewater is used for irrigation of around 10% of crops (Anon 2003). Wastewater irrigated crops show an increased incidence of enteropathogens (Steele and Odemeru 2004). Wachtel *et al.* (2002a) describe *E. coli* contamination of the roots of cabbage irrigated with sewage-contaminated stream water, although the edible part of the plant was unaffected. Islam *et al.* (2004) demonstrated that a single application of *S. Typhimurium* inoculated irrigation water resulted in contamination of carrot and radish at harvest, with *Salmonella* surviving for 203 days in soil postapplication. Lettuce plants irrigated with a single application of *E. coli* O157:H7 contaminated water tested positive for presence of *E. coli* O157:H7 at harvest (30 days postinoculation), and plants contaminated at days 7 and 14 of the study were shown to yield

increased populations (Solomon *et al.* 2003). Quantitative risk assessment models for the use of reclaimed water show that risk varies between crops, with lettuce found to pose a higher risk than cucumber, but comparable to that of broccoli and cabbage (Hamilton *et al.* 2006). The interval between irrigation and harvest will affect the likelihood of pathogenic bacteria surviving to reach the consumer. A survey of UK-based salad vegetable producers showed that over 50% growers will harvest baby-leaf crops within 24 h of the last irrigation (Tyrell *et al.* 2006).

A number of outbreaks have been traced to the use of contaminated water in irrigation. Iceberg lettuce imported from Spain during 2005 caused cases of *S. Typhimurium* throughout the UK and Finland, after wastewater was used to irrigate the crop (Takkinen *et al.* 2005). Cases of *E. coli* O157:H7 in Sweden in 2005 were traced back to lettuce irrigated with water from a stream contaminated with cattle faeces (Söderström *et al.* 2005). Water may also act as a vehicle for the dissemination of viral particles. Beuchat (1996) reports a nontypical outbreak of norovirus linked to celery and irrigation with sewage-contaminated water has resulted in the outbreaks of hepatitis A linked to lettuce consumption (Seymour and Appleton 2001) and spring onions (Josefson 2003).

Hillborn *et al.* (1999) describe an outbreak of *E. coli* O157:H7 attributed to mesclun lettuce, assumed to be irrigated with water contaminated by cattle grazing a nearby field. Solomon *et al.* (2002) showed that *E. coli* O157:H7 in contaminated water can enter the vascular system of lettuce and reach the edible parts of the plant, although the authors point out that unrealistic inoculum concentrations were used.

Soil

Pathogens may be naturally present in soil, for example *Listeria* spp. (Nicholson *et al.* 2005), or may become incorporated in the soil matrix from organic wastes added as fertilizer. Pathogens within soil may contaminate crops directly when heavy rain or water gun irrigation causes leaf splash.

The ability of the pathogen to survive in the environment will impact on the likelihood of crop contamination and pathogen viability at harvest and through to consumption. Initially, the pathogen must survive in the propagation environment until crops are planted out, or in organic wastes applied to the land. Table 2 lists survival times for each enteropathogen from a number of studies.

Survival times are often inconsistent and reflect the variability in propagation environments and organic waste treatments. Kudva *et al.* (1998) demonstrated that aera-

Table 2 Survival time of enteropathogens in the field environment

Pathogen	Environment	Survival (day)	References
<i>Escherichia coli</i> O157:H7	Soil + animal manure	30	Nicholson <i>et al.</i> (2005)
<i>E. coli</i> O157:H7	Soil + animal manure	99	Nicholson <i>et al.</i> (2005)
<i>E. coli</i> O157:H7	Animal manure	60	Avery <i>et al.</i> (2005)
<i>E. coli</i> O157:H7	Slurries	60	Avery <i>et al.</i> (2005)
<i>E. coli</i> O157:H7	Abattoir waste	60	Avery <i>et al.</i> (2005)
<i>E. coli</i> O157:H7	Sewage sludge	60	Avery <i>et al.</i> (2005)
<i>E. coli</i> O157:H7	Nonaerated ovine manure	>365	Kudva <i>et al.</i> (1998)
<i>E. coli</i> O157:H7	Aerated ovine manure	120	Kudva <i>et al.</i> (1998)
<i>E. coli</i> O157:H7	Nonaerated slurry	600	Kudva <i>et al.</i> (1998)
<i>E. coli</i> O157:H7	Aerated slurry	30	Kudva <i>et al.</i> (1998)
<i>E. coli</i>	Slurry + dirty water	90	Nicholson <i>et al.</i> (2005)
<i>Salmonella</i>	Soil	968	Nicholson <i>et al.</i> (2005)
<i>Salmonella</i>	Soil + bovine slurry	300	Nicholson <i>et al.</i> (2005)
<i>Salmonella</i>	Soil + animal manure	30	Nicholson <i>et al.</i> (2005)
<i>Salmonella</i>	Slurry + dirty water	90	Nicholson <i>et al.</i> (2005)
<i>Campylobacter</i>	Soil + animal manure	30	Nicholson <i>et al.</i> (2005)
<i>Campylobacter</i>	Slurry + dirty water	90	Nicholson <i>et al.</i> (2005)
<i>Listeria</i>	Soil + animal manure	30	Nicholson <i>et al.</i> (2005)
<i>Listeria</i>	Slurry + dirty water	180	Nicholson <i>et al.</i> (2005)
<i>L. monocytogenes</i>	Soil + sewage sludge	56	Everis (2004)
Hepatitis A	Water	>365	Seymour and Appleton (2001)
Hepatitis A	Soil	96	Seymour and Appleton (2001)

tion of ovine manure decreased survival of *E. coli* O157:H7 from >365 to 120 days. The application method used for organic wastes may increase survival time: clumping of material applied above ground, and injection application of liquid manures can protect bacteria from desiccation and high temperatures (Hutchison *et al.* 2004).

Stresses encountered during passage through the gut, for example the acidity of the environment, may increase survival by inducing entry to survival stages. *Escherichia coli* and *Salmonella* will exhibit the general stress response, producing a range of stress proteins which can confer cross-resistance to a range of stresses (Barker *et al.* 1999). Cross-protection mechanisms may extend bacterial survival in the environment, by reducing the impact of abiotic factors. Leyer and Johnson (1993) report that after acid adaptation, *S. Typhimurium* displayed increased tolerance of heat and osmotic stress, whilst Hartke *et al.* (1995) demonstrated that pre-irradiation of *Lactococcus lactis* increased resistance to lethal challenges of acid. The stress response of *L. monocytogenes* is similar to that of *E. coli*, but is regulated by the sigma factor σ^B , which has been suggested to increase virulence (Wonderling *et al.* 2004). *Campylobacter* may enter a viable but nonculturable stage (Buswell *et al.* 1998), but the main mechanism of survival is production of large numbers of cells within the host (Jones 2001). Seasonal variation in shedding of pathogens can result in higher than expected microbial loads in faecal material: *Campylobacter* shedding increases

in spring and autumn (Stanley and Jones 2003) and *E. coli* levels during spring and summer (Chapman *et al.* 1997). If increased pathogen loads are present, then simply following guidelines may not be sufficient for preventing crop contamination.

Survival in the phyllosphere

Interest is shifting towards the fitness of the enteropathogen on the leaf surface (phylloplane): if a pathogen can persist on the phylloplane, then the chance of an infectious dose remaining at consumption is increased. Beuchat (1999) showed that *E. coli* O157:H7 contained in bovine faeces and inoculated onto lettuce could be isolated from lettuce up to 15 days after inoculation. Fett (2000) suggested that transient occupants of the leaf, such as enteropathogens, may become incorporated into phylloplane biofilms.

Biofilms are complex structures composed of many species of bacteria, filamentous fungi and yeasts, with 10^6 – 10^8 cells $\text{fw}^{-1} \text{g}^{-1}$ (Morris *et al.* 1998). Cells are enclosed within an exopolymeric matrix, which can buffer environmental changes such as nutrient stress and desiccation (Monier and Lindow 2005); therefore, bacteria within biofilms will have an increased survival rate. Between 30% and 80% of the total bacterial population on a leaf surface will be contained in these aggregates (Morris and Monier 2003), which tend to be associated with sources of nutrients such as leaf veins and glandular

trichomes (Monier and Lindow 2005). Fett (2000) showed that biofilms were present on the cotyledons, hypocotyls and roots of alfalfa, broccoli, sunflower and clover sprouts, by 2-day postgermination.

Enteropathogens can adapt to the phyllosphere environment, but may be outcompeted by epiphytic bacteria (Cooley *et al.* 2006), especially if both species compete for the same carbon source. Interactions between immigrant bacteria and epiphytes are diverse: *Salmonella enterica* has been demonstrated to aggregate with *Pantoea agglomerans* on the leaf surface of cilantro (Brandl and Mandrell 2002) and *Wausteria paucula* was shown to support actively the survival of *E. coli* O157:H7 in the rhizosphere and leaf-surface of lettuce (Cooley *et al.* 2006). Barak *et al.* (2002) demonstrated that *S. Newport* attached to alfalfa sprouts as efficiently as the plant-associated bacteria *Pseudomonas putida*, *Pan. agglomerans* and *Rhanella aquatilis*, and significantly better than *E. coli* O157:H7. However, epiphytes may also limit survival of immigrant bacteria; Cooley *et al.* (2006) demonstrated that *S. Newport* and *E. coli* O157:H7 could be out-competed on lettuce by *Enterobacter asburiae*, repressing growth of the enteropathogens 10-fold. Janiesiewicz *et al.* (1999b) reported that co-inoculation of *E. coli* O157:H7 and *Pseudomonas syringiae* into wound sites on apples suppressed the growth of *E. coli* and Carlin *et al.* (1996) demonstrated that the background flora present on endive prevented the growth of *L. monocytogenes*. These results suggest that there is potential for the naturally occurring microflora to be used as a biocontrol agent, to prevent enteropathogenic bacteria becoming established on the leaf.

Solomon and Matthews (2006) showed that bacterial processes, such as gene expression, motility or production of extracellular compounds, were not necessary for initial attachment but are likely to be important in further colonization and survival on the leaf. Plant-associated bacteria produce acyl-homoserine lactones (AHLs) for communication via quorum sensing, and Brandl (2006) hypothesizes that AHLs may help upregulate factors in enteropathogens beneficial to their survival on the leaf, such as expression of *rpoS* which increases resistance to stresses commonly encountered on the leaf, for example, desiccation.

A major factor in limiting bacterial survival in the phyllosphere is UV radiation. Ecologically successful phylloplane bacteria are efficient in UV-induced DNA damage repair or preferentially colonize sites that are protected from UV, such as within the interior of a leaf (phytopathogens) or at the base of structures such as trichomes (saprophytes) (Jacobs and Sundin 2001). The biofilm matrix also shields against the damaging effects of UV irradiation (Elasri and Miller 1999). Phyllosphere communities exhibit a marked shift towards UV tolerant

phenotypes, for example, pigmented bacteria, as the growing season progresses (Jacobs and Sundin 2001). In *Pseudomonas syringae*, expression of the gene *rulAB* confers DNA repair capabilities and therefore increased UV tolerance (Sundin and Murillo 1999). *Escherichia coli* and *S. enterica* possess homologues of *rulAB* (Brandl 2006), suggesting an ability to withstand UV irradiation.

Enteropathogens encounter osmotic stress frequently when passing through the host gut, and consequently display a number of stress-avoidance mechanisms, mediated by *rpoS*, which may induce cross-resistance to stresses encountered on the leaf (Brandl 2006). For this to impact on survival, pathogens would have to become established on the leaf surface relatively and quickly after excretion from the host. Exposure to plant-produced antimicrobials upregulates a homologue of the sap operon in *Erwinia chrysanthemi*. In *S. enterica*, induction of the sap operon promotes acid-resistance and therefore survival in the acidic conditions of the gut; Brandl (2006) suggests that therefore a period of residence in the phyllosphere may lead to increased virulence of enteropathogens.

Further protection from environmental stresses may be afforded by movement into the internal tissue of the plant. This is normally a passive process, unlike the destructive entry of many phytopathogens: enteropathogens in irrigation water can be taken up by the root systems and enter the edible portion of the crop (Wachtel *et al.* 2002a), for example, lettuce (Seo and Frank 1999; Takeuchi and Frank 2000; Solomon *et al.* 2002), apple (Burnett *et al.* 2000) and tomato (Guo *et al.* 2001, 2002). Enteropathogens may also gain entry via wounds (Janiesiewicz *et al.* 1999), or structures such as lenticels (Burnett *et al.* 2000) and stomata (Seo and Frank 1999). Infiltration of enteropathogenic bacteria through these structures can occur when bacteria are present in water on the surface of fruits (Burnett *et al.* 2000) and can be increased during processing if wash water is of a lower temperature than the fruit, creating a negative temperature differential (Buchanan *et al.* 1999).

The presence of phytopathogens may increase the penetration and growth of enteropathogenic bacteria, because of disruption of the cuticle and increased release of nutrients (Wells and Butterfield 1999). Richards and Beuchat (2005) report that co-inoculation of wound sites on cantaloupe with *S. Poona* and the phytopathogens *Cladosporium cladosporioides* or *Penicillium expansum* increased penetration of *Salmonella* into the internal tissues of the fruit, because of the tissue breakdown caused by the fungi. Stopforth *et al.* (2004) demonstrated that *E. coli* O157:H7 can survive and proliferate in injured apple tissue, even after the use of sanitizers.

Oron *et al.* (1995) demonstrated that poliovirus applied to the roots of tomato plants can be recovered from the

leaves, but not the fruit. This was attributed to the presence of antiviral substances and not to an inability of the virus to reach the fruit. Dingman (2000) analysed the proliferation of *E. coli* O157:H7 in bruised apple tissue and observed that growth was suppressed in McIntosh apples, unlike the other cultivars used. This was thought to be due to production of an unstable or volatile inhibiting factor, as the effect was reduced during storage. Reinders *et al.* (2001) studied the effect of caffeic acid on *E. coli* O157:H7 survival in a model apple juice medium and demonstrated a reduction in *E. coli* populations, suggesting that phenolic acids play an important role in limiting the bacterial survival *in planta*. A range of phenolic acids, including caffeic acid, were shown to inhibit the survival of *L. monocytogenes in vitro* (Wen *et al.* 2003) and Delaquis *et al.* (2006) report evidence that an antilisterial factor, thought to be of a phenolic nature, is produced by wounding (shredding) of iceberg lettuce and therefore is likely to play a role in limiting *L. monocytogenes* growth in bagged salads.

The interaction between the host plant and epiphytes, symbionts and phytopathogens has been extensively studied. However, the role of plant-microbe interactions in limiting the colonization by enteropathogenic bacteria is not so well described. Production of antimicrobial factors may be a direct response to the presence of pathogenic bacteria. Dong *et al.* (2003) suggest that genetic aspects of both the host plant and *Salmonella* are involved in endophytic colonization. Barak *et al.* (2004) showed that virulence factors, including aggregative fimbriae and expression of *rpoS*, were involved in *S. enterica* attachment to alfalfa. Interestingly, these virulence genes are essential for infection in an animal host. There are a number of similarities between the systems employed by plant and animal pathogens. The type III secretion system (TTSS), which enables the delivery of pathogenicity proteins to the host cell, is conserved across the Gram-negative plant and animal pathogens, although the proteins secreted differ (Hueck 1998). Recognition of elements of the TTSS in phytopathogens causes induction of host plant defence mechanisms (Hueck 1998). The conserved nature of these factors in human pathogens suggests that plants may also respond to the presence of enteropathogenic bacteria in the phyllosphere. Iniguez *et al.* (2005) describe the role of host defences in limiting endophytic colonization by the enteric bacterium, *S. Typhimurium*. Addition of ethylene, the signal molecule which induces systemic resistance, decreased *Salmonella* colonization of the roots of the legume *Medicago trunculata*. Use of *Salmonella* mutants, deficient in structural components of the TTSS or flagella, showed increased colonization of the roots of alfalfa, and ethylene production was reduced, suggesting that the plant did not recognize the pathogen.

Further colonization studies using an *Arabidopsis thaliana* mutant (*npr1*) provided evidence that recognition of TTSS components induces salicylic acid-mediated defence signalling (Iniguez *et al.* 2005). The authors suggest that overexpression of defence-related genes in crop plants may present a novel method to control enteropathogen colonization in the field.

Conclusions

There is a dichotomy between POS survey data and outbreak instance. Overall, surveys suggest a low incidence of contamination. The regulations imposed on growers and the demand for comprehensive hazard analysis and critical control point (HACCP) strategies should prevent any environmental contamination reaching the consumer; however, these approaches do not fully prevent contamination remaining at POS. The fact that outbreaks linked to fruit and vegetable products can and do occur indicates that these outbreaks are the result of occasional contamination events, which are difficult to identify and control. At present, the food industry relies on postharvest interventions to limit the number of enteropathogens present on fresh produce at POS. The use of sanitizers, especially chlorine-based products, is an area of media interest and consumer concern, and chemicals cannot be used in organic production. Prevention of contamination therefore is a preferred strategy. To influence the survival of bacteria in the phyllosphere, we need to better understand the behaviour of bacteria on the leaf-surface, their interactions with other microbes and the host plant and the potential for internalization. Improved understanding of these areas will lead to new methods to limit enteropathogen survival on food crops. Potential areas of exploitation could include the addition of competitive microflora, the use of quorum-sensing molecule analogues to disrupt bacteria communication, or approaches aimed at preventing attachment, which could be targeted specifically at the molecular mechanisms involved. Further information on the factors which can limit survival in the field may lead to potential new intervention strategies to maximize the impact on bacterial communities, either on the plant itself or in water, soil and organic fertilizers prior to application, for example improved treatment methods or modified propagation environments.

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