2017

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CHAPTER 17
Microbial ecology of fresh vegetables

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The total consumption of fresh and processed vegetables has exceeded 123 kg per capita in the United States and 118 kg per capita in the European Union (http://www.helgilibrary.com/indicators/index/vegetable-consumption-per-capita) in 2009. Changes in lifestyles and consumption trends have prompted the sustained growth of fresh-cut or minimally processed vegetables that are fresh and ready-to-eat (RTE). In 2011, for example, total US fresh-cut produce sales through both food service and retail channels were estimated to surpass $27 billion (Cook et al., 2012).

17.1 Introduction

The incidence of food-borne illness outbreaks caused by contaminated fresh and fresh-cut vegetables has increased globally in recent years. In the US, an estimated 34% of all food-borne illnesses that led to hospitalization or death, from 1998 to 2008, were attributable to vegetables (Painter et al., 2013). Among these, contaminated leafy vegetables were the leading causes (22%), followed by vine-stalk (7.9%), and other commodities. The pathogens most frequently linked to vegetable-related outbreaks include bacteria (Salmonella, Escherichia coli) and viruses (norovirus, hepatitis A) (Painter et al., 2013). As an example, hot peppers were the source for a high profile salmonellosis outbreak in the US and Canada in 2008, leading to 1442 illnesses and two deaths (Mody et al., 2011). In addition, contaminated pre-packaged baby spinach caused a devastating E. coli O157:H7 outbreak in 2006 in the US, involving 199 cases and three deaths (CDC, 2006). In the wake of these food-borne illness outbreaks, research has begun in earnest to define the complex but critical biological interactions among indigenous microorganisms, human pathogens, and fresh produce. In this chapter, an in-depth review of the microbial ecology of fresh and fresh-cut vegetables and their relationship to the major food-borne bacterial pathogens is presented.

17.2 Prevalence and diversity of microbial communities on fresh vegetables (post-harvest)

Vegetables are known to harbor a diverse and complex array of bacterial communities. Coliforms and fecal coliforms, like generic E. coli, are considered indictors of fecal contamination and their presence on food suggests that sanitary quality might be compromised. In 2009, The Consumer Union tested 16 different brands of salad greens (n = 208 bags) collected in the New York City Metro area and found about a third of the bags tested had more than 10,000 cfu/g of total coliforms and approximately 5% of the bags contained generic E. coli (Consumer Union, 2010). A Canadian-based group conducted a similar retail-level study...
across five provinces involving imported herbs and pre-packaged leafy greens and spinach from five countries. The highest prevalence of coliforms were found in imported US leafy greens (mean of 21 samples (66.7% of total tested) – 3.3 log_{10} cfu/g) followed by US herbs (mean of 12 samples (7% of total tested) – 2.6 log_{10} cfu/g) (Allen et al., 2013). Although not currently used in the US, hygienic standards for minimally processed vegetables exist in other countries. For example, the European Union guidelines are 100 cfu/g generic *E. coli* in RTE pre-cut fruits and vegetables. A recent field survey of both organically and conventionally grown lettuce grown in Spain found that 20% of samples exceeded this level (i.e., 35% of samples collected tested positive) (Oliveira et al., 2010).

Pathogenic bacteria (e.g., *Listeria monocytogenes*, *Escherichia coli*, *Salmonella*) can also be part of larger microbial communities on fresh produce (Shi et al., 2009; Teplitski et al., 2011). Field surveys on contamination of produce with pathogenic bacteria such as *E. coli* O157:H7, *Salmonella* spp., and *L. monocytogenes* found drastically different results depending on the methods, location, seasons, produce type, and criteria of the survey conducted. Some revealed little or no occurrence of contamination with these pathogenic bacteria (Consumer Union, 2010; Koseki et al., 2011; Sant’Ana et al., 2011; Allen et al., 2013; Althaus et al., 2012), while others showed widespread contamination. Salleh et al. (2003) examined 112 samples from four local salad vegetables in Selangor, Malaysia, and found 40 (35%) were contaminated with *Salmonella* with a total of 31 different *Salmonella* serovars being isolated from this study. In Spain, *Salmonella* spp. were detected in 0.7% of lettuce samples (*n* = 137) (Sospedra et al., 2013). In 2014, Wijnands et al. (2014) conducted a survey to estimate pathogen prevalence and contamination levels of raw produce as well as resulting minimally processed packaged salad as sold in the Netherlands. The overall prevalence for *L. monocytogenes*, *E. coli* O157, and *Salmonella* was 0.11, 0.11, and 0.38%, respectively, across 1800 samples of produce and 1900 samples of RTE mixed salads investigated. Prevalence point estimates for *Salmonella* in specific produce ranged from 0.53% in iceberg lettuce to 5.1% in cucumber. In the Philippines (Vital et al., 2014), 24.7% of retail fresh produce samples, including bell pepper, cabbage, carrot, lettuce, and tomato, were positive for *Salmonella* spp. It is noteworthy that all of these studies indicated that a wide range of vegetables can be contaminated by pathogenic bacteria and are potentially capable of serving as vehicles for human infection. However, manifestation of these capabilities can be greatly influenced by intrinsic and extrinsic ecological factors naturally present in produce or imposed at one or more points during pre-harvest and post-harvest process lines.

### 17.3 Post-harvest persistence, colonization, and survival on fresh vegetables

Numerous studies have examined the growth or die-off of pathogens under variable temperature conditions experienced during the processing, storage, and shipping of fresh-cut or minimally processed vegetables. Hard conclusions of growth rates and maximum population densities based upon these individual investigations are difficult due to variances in experimental design, test strains (including the use of stressed versus non-stressed bacterial cells), produce type, and distinct packaging materials. It is an accepted fact that native bacterial populations, including any pathogens that might be present, are in constant flux during post-harvest handling and storage. Moreover, variables such as storage temperature and time greatly impact microbial levels and product quality. The US Food and Drug Administration Food Code requires that packaged ready-to-eat fruits and leafy green vegetables be refrigerated at less than or equal to 5°C to minimize the growth of food-borne pathogens. Luo et al. (2009) noted increases in native microflora and *E. coli* O157:H7 after storage at ≥8°C within the labeled “Best If Used By” date of bagged baby spinach. In addition, *E. coli* has been noted to grow on
fresh-cut iceberg lettuce an additional 1 log cfu/g over a period of 6 hours at temperatures above 16 °C (Rodríguez-Caturla et al., 2012). Similarly, multiple studies have demonstrated Salmonella’s ability to persist on whole produce, internally and externally, across a broad range of temperatures from 4 to 25 °C for 7 days up to 8 weeks (Liao et al., 2010; Vandamm et al., 2013; Shi et al., 2007; Iturriaga et al., 2007; Kroupitski et al., 2009; Zhou et al., 2014; Beuchat and Mann, 2008). Higher storage temperatures (above 15 °C) and longer storage time typically allow Salmonella to grow on or within a produce commodity. An examination of growth kinetics (Table 17.1) of Salmonella Newport growth on beefsteak and Roma tomatoes shows no significant difference in growth between these two tomato types, and after a lag phase of approximately 6h, the exponential growth rate reaches almost 0.3 log/h. Pre-storage of the tomatoes at 5 °C did not alter the growth kinetics compared to pre-storage at 22 °C (Table 17.2), suggesting that pre-storage temperature has little impact on the growth of Salmonella once it is held at a higher temperature for storage after slicing. Conversely, storage at lower temperatures (lower than 10 °C) suppresses Salmonella growth, and with longer storage times, a decrease in cell number may occur (Pao et al., 2012; Vandamm et al., 2013).

Luo and others (2010) looked at fresh-cut romaine and iceberg lettuce inoculated with E. coli O157:H7 and resealed in bags containing the original O2 levels. At 5 °C, E. coli O157:H7 populations decreased almost 2 log cfu/g by day 10 even though viable cells were still detected. McKellar et al. (2012) monitored and recorded the temperatures of 27 cases of packaged lettuce throughout various stages of storage and shipping from the processor to the retail shelf of three stores. The results indicate a nearly 1 log reduction in viable bacterial cells and the extent of die-off was proportional to the overall time spent in refrigeration. The authors suggested a reduced risk of illness to consumers when fresh-cut lettuce is stored at 5 °C or below prior to consumption. L. monocytogenes, on the other hand, is psychrotropic and capable of growth at low temperatures due to a variety of intrinsic physiological attributes (Laksanalamai et al., 2011).

Several studies examined the growth potential of L. monocytogenes in a variety of fresh produce (Ells and Truelstrup, 2006; Sant’Ana et al., 2012, 2013; Skalina and Nikolajeva, 2010; Tian et al., 2012). In all of these commodities,

### Table 17.1 Growth kinetics of S. Newport in fresh-cut red ripe tomatoes stored at 22 °C.

<table>
<thead>
<tr>
<th></th>
<th>pH</th>
<th>a_w</th>
<th>LDT (h)</th>
<th>EGR (log/h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beefsteak</td>
<td>4.25</td>
<td>0.994</td>
<td>6.40 ± 0.90</td>
<td>0.299 ± 0.010</td>
</tr>
<tr>
<td>Roma</td>
<td>4.18</td>
<td>0.994</td>
<td>5.77 ± 0.49</td>
<td>0.298 ± 0.014</td>
</tr>
</tbody>
</table>

### Table 17.2 Effect of storage temperature before cutting on the growth kinetics of S. Newport inoculated in fresh-cut red ripe tomatoes stored at 22 °C.

<table>
<thead>
<tr>
<th>Storage temperature</th>
<th>22 °C</th>
<th>5 °C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LDT (h)</td>
<td>EGR (log/h)</td>
</tr>
<tr>
<td>Beefsteak</td>
<td>2.28 ± 0.95</td>
<td>0.232 ± 0.029</td>
</tr>
<tr>
<td>Roma</td>
<td>2.46 ± 0.73</td>
<td>0.251 ± 0.006</td>
</tr>
</tbody>
</table>
*L. monocytogenes* grew 1–2 logs in 7–14 days at 5°C. Growth of 3–4 logs was noticed, however, when the temperature was increased to 10–15°C. In a detailed commodity-specific study of *L. monocytogenes*, the growth potentials of *L. monocytogenes* strains were examined on fresh-cut celery at different temperatures and incubation periods. A cocktail of three outbreak strains of *L. monocytogenes* including serotypes 1/2a, 1/2b, and 4b were used as an inoculum at $3 \times 10^3$–$5 \times 10^3$ cfu per 5 g of celery. The inoculated samples were stored at 5°C for 30 days, 10°C for 12 days, and 25°C for 7 days. The growth pattern of all three serotypes was comparable at all temperatures; the growth rate was found to be much slower at 5°C followed by 10 and 25°C. The increase in counts at the end of the sampling periods were approximately 3–4 log cfu at 25°C, 2 log cfu at 10°C and 1 log cfu at 5°C per 5 g of celery (Sahu et al., 2014; see Table 17.3). Maximum growth was found to be primarily a function of temperature and duration of incubation while the growth rate was dependent solely on the temperature of storage. These results clearly indicated that *L. monocytogenes* could survive and grow on a variety of fresh vegetables even at refrigerated temperatures. Zeng and others (2014) conducted a comprehensive large-scale study in the US placing temperature sensors in cross-country transport trucks, as well as in the storage rooms and display cases in nine supermarkets to determine temperature fluctuations that fresh-cut bagged leafy greens might experience during a one to three day storage period in each location prior to purchase by the consumer. Temperature profiles were then reproduced with *E. coli* O157:H7 and *L. monocytogenes* artificially inoculated on to romaine lettuce. Retail storage showed the largest temperature fluctuations (i.e., range of 0.6 to 15.4°C) and, correspondingly, the levels of *E. coli* O157:H7 and *L. monocytogenes* showed the greatest increase, around 3.0 log cfu/g, over the three days of storage. Mean measured temperatures for transport and display in retail cases were rarely above 6°C. While *E. coli* O157:H7 growth was essentially static during this time, *L. monocytogenes* barely proliferated under transport conditions ($\leq 0.6$ log cfu/g) but increased up to 1.1 log cfu/g during three days of housing in display cases (Zeng *et al*., 2014). This study illustrated the importance of maintaining an uninterrupted cold chain.

Temperature abuse has not only been noted to occur within the retail environment but can also be a problem within the consumer’s home. Somewhat alarming was the notion made by James *et al.* (2008) who reviewed 20 published studies, conducted in parts of Europe, the US, and New Zealand from 1987 to 2006, and found only two studies reporting mean air temperatures of less than 5°C in domestic refrigerators. Most studies showed a great deal of concordance, citing temperatures in >50% of refrigerators above 5°C.

The pH of a produce commodity may also contribute to the ability of pathogens to grow on

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**Table 17.3 Growth of Listeria monocytogenes in artificially contaminated cut celery.**

<table>
<thead>
<tr>
<th>Strain</th>
<th>5°C</th>
<th>10°C</th>
<th>25°C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Maximum growth (days)</td>
<td>Doubling time (h)</td>
<td>Maximum growth (days)</td>
</tr>
<tr>
<td>LS806(4b)</td>
<td>$2.6 \times 10^4$ (30 d)</td>
<td>87.6</td>
<td>$1.2 \times 10^5$ (12 d)</td>
</tr>
<tr>
<td>LS814(1/2a)</td>
<td>$2.7 \times 10^4$ (30 d)</td>
<td>72.8</td>
<td>$1.7 \times 10^5$ (12 d)</td>
</tr>
<tr>
<td>LS810(1/2b)</td>
<td>$4.3 \times 10^4$ (30 d)</td>
<td>74.1</td>
<td>$2 \times 10^6$ (12 d)</td>
</tr>
</tbody>
</table>
it. For example, the optimal pH range for *Salmonella* is 6.5–7.5. However, the pH range in which salmonellae can survive and grow is much broader (i.e., 3.7–9.5). Tomatoes are considered to be relatively acidic with pH values ranging from 4.37 for round to 4.67 for grape tomatoes (Beuchat and Mann, 2008), but these values are not outside of the pH range to prevent the growth of *Salmonella*. As shown in Table 17.1, *S. Newport* was able to grow in both red round and Roma tomatoes with a pH of 4.2. Inspecting the growth kinetics of *S. Newport* in blended tomatoes with pH adjusted from 3.9 to 4.3 (Table 17.4), growth was observed at all pH levels, albeit at a lower rate and at the lowest pH. This low pH tolerance was also observed by Asplund and Nurmi (1991), where three different *Salmonella* serovars, Enteritidis, Infantis, and Typhimurium, were all able to grow on cut tomatoes with low pH values (3.99–4.37). The ability of *Salmonella* to grow to such high numbers in tomatoes may be due to the major acidulants within tomatoes, citric and malic acids, to which salmonellae may be more tolerant.

Packaging may affect the growth of pathogens on fresh-cut vegetables as well. Moisture enters bags of fresh-cut leafy greens as a residual from product washing. Vapor condensation could also lead to the accumulation of water inside the bags. Valentin-Bon *et al.* (2008) suggested that moisture (i.e., condensation) observed at the bottom of many bags may provide a better opportunity for microbial growth including that of any pathogens present. In a subsequent study, Kase *et al.* (2012) noted an absence of moisture condensation in the bags, which may suggest industry advances in moisture control and bag design (e.g., anti-fog film, perforated versus non-perforated, etc.).

Differences in genetic make-up and efficiency in expression of stress-related pathways most likely dictate the ability of a particular serovar or strain to survive when exposed to various stressors. For example, *Salmonella* possesses the ability to survive in a desiccated state. The desiccation tolerance of several different serovars demonstrated a range of survival from 36 to 80%, depending on the serovar. Additionally, exposing salmonellae to desiccation conditions induced tolerance to several other stressors, including high salts, ethanol, bleach, high temperatures, and UV irradiation (Gruzdev *et al.*, 2011).

### 17.4 Routes of contamination during post-harvest handling of fresh and fresh-cut vegetables

During post-harvest processing, various routes of contamination with human pathogens may include contaminated water used for washing, chill tanks or sprays and shipping ice, processing equipment and transportation, infected workers, and cross-contamination from food preparation, display, and storage. Recently, Johnston *et al.* (2005) studied the quality of fresh produce at different stages from harvest throughout the packing shed in the southern United States. The group found that total aerobic plate count (APC) levels in cilantro increased from the field and throughout packing, with mean ranges of 5.7 log in the field to 6.7 log cfu/g in the samples obtained from boxes ready for distribution. Total coliforms increased significantly from harvest through packing, with a peak occurring mainly at the rinse step. All of these results suggested that microbial contamination could either increase or originate during post-harvest processing. Several key areas have been identified as high risk for

<table>
<thead>
<tr>
<th>pH</th>
<th>LDT (h)</th>
<th>EGR (log/h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.3</td>
<td>5.32 ± 0.87</td>
<td>0.189 ± 0.065</td>
</tr>
<tr>
<td>4.2</td>
<td>4.92 ± 3.47</td>
<td>0.105 ± 0.088</td>
</tr>
<tr>
<td>4.1</td>
<td>6.58 ± 3.11</td>
<td>0.167 ± 0.096</td>
</tr>
<tr>
<td>4.0</td>
<td>7.57 ± 2.73</td>
<td>0.146 ± 0.105</td>
</tr>
<tr>
<td>3.9</td>
<td>6.9 ± 2.10</td>
<td>0.123 ± 0.024</td>
</tr>
</tbody>
</table>

*pH adjusted with citric acid.*
cross-contamination including water used to wash produce, kitchen surfaces, cutting surfaces, and worker hand hygiene, including the use of gloves (Doyle and Erickson, 2008; Todd et al., 2010; Waitt et al., 2013; Zhou et al., 2014).

Water washes are often used immediately after harvest to remove field debris before subsequent processing steps. In the tomato industry, tomatoes are brought from fields to packing houses where they are placed in large dump tanks for washing before sorting and sizing. Contamination of this water can lead to internal and external contamination of the tomatoes. Internal contamination occurs when water and other foreign material, such as Salmonella, rush into the tomato via the stem scar due to differences in hydrostatic pressure, differences in temperature, or capillary action (Bartz, 1982; Smith et al., 2006). Recent findings have demonstrated that even with a 10 °F positive temperature differential (i.e., washwater 10 °F warmer than incoming tomatoes) Salmonella was still able to internalize (Zhou et al., 2014).

Common kitchen surfaces are another mechanism for transfer of pathogens to fresh produce. In one large study examining many common kitchen surfaces, such as ceramic, glass, plastic, and stainless steel, it was demonstrated that Salmonella can readily be transferred from contaminated surfaces to fresh produce, with higher transfer rates from wet surfaces (79–97% transfer) (Jensen et al., 2013). The potential for fresh produce to contaminate the kitchen surface also exits, albeit at significantly lower rates of transfer. This directional tendency of pathogens to move from the kitchen surface to produce is attributed to the limited availability of nutrients and suitable attachment sites on abiotic surfaces. Additionally, microbial attachment to produce commodities is facilitated by the presence of complex carbohydrates (Jensen et al., 2013). S. Montevideo was transferred from the surface of a tomato to the interior by cutting with a sterile knife (Lin and Wei, 1997). The amount transferred and the depth of transfer into the tomato pulp increased in a dose-dependent manner. Additionally, when the same knife was used to cut subsequent tomatoes, transfer was noted well into the interior of the next tomato. A key example of cutting surface involvement leading to an outbreak situation happened in Queensland, Australia, where an outbreak of S. Bovismorbificans linked to cut lettuce leaves occurred. Investigators discovered the outbreak organisms on the surface of the cutting equipment used to process the lettuce and sited inadequate cleaning and sanitation of the cutting equipment leading to the contamination of lettuce products (Stafford et al., 2002). Listeria has been isolated in several food processing environments. Listeria, specifically L. monocytogenes, has the ability to grow on different food contact surfaces where it is capable of establishing biofilms (Silva et al., 2008). The organism has been isolated from various locations on food premises including drains, abattoirs, conveyor belts, freezers, smoke houses, slicing blades, packaging machines, floors and walls, footbaths, air ducts, and others (Moretro and Langsrud, 2004; Sofos, 2008). Biofilms can protect the embedded bacteria from antibacterial treatments such as sanitization, desiccation, UV radiation, concentrated disinfectants, etc., allowing the persistence of L. monocytogenes for long periods of time in the processing environment. These biofilm-coated surfaces become sources of frequent contamination when food products come into contact with them (Carpentier and Cerf, 2011; Hall-Stoodley et al., 2004).

Good worker hygiene is critical to prevent the transfer of pathogens to fresh produce as well. The use of gloves as a barrier can be an effective means to reduce the risk of transfer. Unfortunately, glove use can lead to a false sense of security and unsafe practices are adopted because workers believe that gloves will prevent any cross-contamination (Todd et al., 2010). Gloves were shown to aid in the transfer of S. Enteritidis to the edible portions of living lettuce during harvest (Waitt et al., 2013). Moreover, high transfer rates were seen from the point of contamination to the subsequent three heads of
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17.5 Microbial adaptation on produce commodity

It has become clear that natural selection plays a significant role in the adaptive change now observed among Salmonella strains associated with the food supply, produce notwithstanding. The recent application of whole-genome sequencing, with both fragment-based and long read technologies included, has yielded important clues into the specific genetic determinants that give rise to adapted phenotypes in Salmonella. Moreover, these data have provided substantial insight into the genetic mechanisms that underpin adaptive change among salmonellae now thriving in produce and other post-harvest processing niches including the mobilome (i.e., high-frequency horizontally transferrable elements such as phage, transposable elements, and other recombination hotspots across the genome) of Salmonella. The intersect of adaptive change and horizontal transfer is not insignificant as it has been postulated that those changes which are honed by selection are then transferred rapidly across the population by means of laterally enhanced regions of the genome (Allard et al., 2012).

Adaptation among Salmonella strains may account for the novel genetic changes now emerging among certain produce vehicles. S. Saintpaul, for instance, recently associated with one of the largest fresh produce contamination events ever documented in North America, appears to have acquired a number of nucleotide substitutions that distinguish it from other non-tomato/pepper associated Saintpauls. A recent study by Hayford et al. (2015) (Figure 17.1) documents a variety of non-synonymous single nucleotide changes across the genome when compared to other isolates from other produce-and non-produce-related sources. Interpretation of these nucleotide substitutions in the context of the amino acid PAM matrix pointed to several amino acid changes with functionally relevant differences in the host protein (Mount, 2008). That is, several non-synonymous changes were conserved biochemically and may play a role in the enhanced survival of S. Saintpaul strains in pepper and tomato and potentially in other members of the Solanaceae. Surprisingly, these changes have provided a substantial clue to potential areas of adaptation having occurred in genes responsible for propanediol utilization (pduF) and propanediol diffusion facilitator (pudB) genes. Interestingly, propanediol is a metabolite resulting from ripening or rotting of plant tissues (Bobik et al., 1997; Brandl et al., 2013; Goudeau et al., 2013). Previous studies revealed that populations of mutants deficient in propanediol utilization were several logs lower than wild-type strains grown in cilantro (Goudeau et al., 2013). In the light of S. Saintpaul’s documented association with at least Jalapeno and Serrano peppers, these changes may be of significance, representing key adaptive changes among produce-specific Salmonella. Moreover, these changes may signal

![Propanediol Operon](image)

**Figure 17.1** Genetic Map of Salmonella Saintpaul propanediol operon (see GenBank sequence gb|AOXY01000026.1). Regions of two propanediol genes (pduF and pduB) with location and positions of nonsynonymous and synonymous SNP mutations.
emerging alleles in produce niches such as jalapeno peppers, yielding an adaptive metabolic premium allowing for survival of specific S. Saintpaul in harsh and relatively narrow produce settings.

Adaptive change among bacteria in the produce and produce-processing environment is likely to be further driven by intrinsic genetic factors known to enhance evolutionary change and the acquisition of adaptive change among enteric pathogens. Such evolution may be explained in part by the hypermutable phenotype (LeClerc et al., 1996) caused by defects in the bacterial methyl-directed mismatch repair (MMR) system. Up to 73% of the MMR defects found in feral settings are due to lesions within the mutS gene, resulting in increased nucleotide substitution rates, enhanced DNA transposition, and, perhaps most importantly, a relaxation of the internal barriers that normally restrict homologous recombination following horizontal gene transfer (HGT) of foreign DNA (LeClerc and Cebula, 1997). The now incontrovertible connection between HGT and MMR gene evolution has led to the thesis that genetic exchange of mutS alleles could simultaneously quiet the mutator phenotype while rescuing adaptive changes from the population (LeClerc et al., 1996; Brown et al., 2001). Consistent with this hypothesis, the mutS gene is evolutionarily scrambled by HGT in subspecies I S. enterica, which has been documented in our laboratories (Brown et al., 2002, 2003).

In L. monocytogenes, two cell-cell communication systems, luxS orthologous (Challan et al., 2006) and virulence regulator, the arg system (Riedel et al., 2009; Rieu et al., 2007), have been reported to be involved in the regulation of biofilm formation. luxS mutants in L. monocytogenes strains are reported to form denser biofilms than parental strains (Sela et al., 2006). Flagellum-mediated motility also plays an important role in biofilm formation in L. monocytogenes (Lemon et al., 2007). Virulence genes like prfA and inlA are also reported to have important roles in biofilm development in L. monocytogenes. The prfA mutant is reported to present a defective biofilm compared with the wild type (Lemon et al., 2010), whereas truncated InlA showed enhanced biofilm formation compared with the full length one (Franciosa et al., 2009). It is interesting to note that many proteins in L. monocytogenes have evolved to serve both for environmental adaptation such as biofilm formation and adaptation in human hosts leading to disease manifestation.

17.6 Effective post-harvest intervention technologies

The produce industry faces unique challenges for eliminating pathogen contamination when compared to other types of foods. In 2013, the US FDA Food Safety Modernization Act (FSMA) proposed rules for produce safety to set standards in identified routes of microbial contamination of produce, including: (1) agricultural water; (2) biological soil amendments of animal origin; (3) health and hygiene; (4) animals in the growing area; and (5) equipment, tools, and buildings (http://www.fda.gov/Food/GuidanceRegulation/FSMA/ucm334114.htm). However, even with the proper plan and systems in place, it may still be possible for some microbial contamination to occur.

A better understanding of microbial ecosystems on the surface of raw vegetables would be extremely useful when developing interventions to minimize contamination, prevent the growth of pathogens, and kill or remove pathogens at various stages of pre- and post-harvest. The composition and abundance of microbial ecosystems unique to various types of produce (Leff and Fierer, 2013; Barak et al., 2008) can be greatly influenced by changes in practice and field conditions prior to harvest and alterations in conditions of various stages after harvesting.

After weeks of sun and ambient temperature exposure, vegetables typically undergo a pre-cooling step soon after harvest to reduce the field heat. Pre-cooling is generally done through forced air cooling, hydrocooling (cold water
Table 17.5 Chemical and physical interventions on fresh and fresh-cut vegetables in post-harvest.

<table>
<thead>
<tr>
<th>Disinfection technique</th>
<th>Dose/concentration</th>
<th>Investigated vegetable commodity</th>
<th>Targeted microorganism</th>
<th>Results</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Chemical-based disinfection</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chlorine dioxide</td>
<td>Aqueous: 100 ppm Gaseous: 1.2–4.1 mg/l</td>
<td>Cucumber, lettuce, carrot, tomato, onion, and cabbage</td>
<td>Bacteria, yeast, molds</td>
<td>1.5–5.8 log reduction</td>
<td>Singh et al. (2002), Chung et al (2011), Sy et al. (2005)</td>
</tr>
<tr>
<td>Organic acids</td>
<td>0.5–1.0%</td>
<td>Iceberg lettuce, spinach</td>
<td>Bacteria</td>
<td>1–2 log reduction</td>
<td>Akbas and Olmez (2007), Neal et al. (2012)</td>
</tr>
<tr>
<td>Hydrogen peroxide</td>
<td>1–3%</td>
<td>Mushrooms, tomatoes, red bell peppers, lettuce, spinach, cucumbers, zucchini, and bell peppers</td>
<td>Bacteria</td>
<td>1–3 log reduction</td>
<td>Back et al. (2014), Moore et al. (2011), Kim et al. (2007), Sapers and Simmons (1998)</td>
</tr>
<tr>
<td>Peroxyacetic acid</td>
<td>80–100 ppm</td>
<td>Lettuce, spinach, celery, cabbage, and leek</td>
<td>Bacteria, yeast, molds, and virus</td>
<td>1–2 log reduction</td>
<td>Neal et al. (2012), Fraisse et al. (2011), Vandekinderen et al. (2009)</td>
</tr>
<tr>
<td>Calcium-based solutions</td>
<td>1.5%</td>
<td>Lettuce</td>
<td>Bacteria</td>
<td>1–2 log reduction</td>
<td>Martin-Diana et al. (2005)</td>
</tr>
<tr>
<td>Ozone</td>
<td>Up to 10 ppm O₃</td>
<td>Lettuce, potato, carrot, spinach, cucumber, tomato, baby leaf, brassica, cabbage, rocket leaf, pepper</td>
<td>Bacteria, yeast, molds, and fungus</td>
<td>Up to 2.6 log reduction</td>
<td>Singh et al. (2002), Garcia et al. (2003), Beltran et al. (2005a, 2005b), Horvitz and Cantalejo (2014)</td>
</tr>
<tr>
<td>Plant extracts</td>
<td></td>
<td>Cilantro, parsley, spinach, carrot, and tomato</td>
<td>Bacteria</td>
<td>Up to 4 log reduction</td>
<td>Singh et al. (2002), Orue et al. (2013), Lu and Wu (2010), Mattson et al. (2011)</td>
</tr>
<tr>
<td><strong>Physical-based treatment</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thermal treatments</td>
<td>85–100 °C</td>
<td>Lettuce, green bell pepper</td>
<td>Bacteria, yeast, molds and parasite</td>
<td>1 log reduction</td>
<td>Rico et al. (2007), Duhain et al. (2012)</td>
</tr>
<tr>
<td>High-pressure processing</td>
<td>300–600 mPa</td>
<td>Tomato</td>
<td>Bacteria</td>
<td>0.5–3.6 log reduction</td>
<td>Maitland et al. (2011)</td>
</tr>
<tr>
<td>Irradiation</td>
<td>≤1.0 kGy</td>
<td>Cilantro, lettuce</td>
<td>Bacteria, molds, yeast</td>
<td>Up to 6.7 log reduction</td>
<td>Foley et al. (2004), Goularte et al. (2004)</td>
</tr>
<tr>
<td>Ultraviolet light</td>
<td>1.18 kJ/m², 2.37 kJ/m²</td>
<td>Lettuce</td>
<td>Bacteria, yeast, and molds</td>
<td>1–2 log reduction</td>
<td>Allende et al. (2006)</td>
</tr>
</tbody>
</table>
dunk or rinse), icing (direct or indirect contact with produce), vacuum cooling, or hydrovac cooling (i.e., water is sometimes sprayed on the produce prior to vacuum cooling). Vegetables in close contact with the ground usually undergo a washing step following harvest to remove dirt and other debris taken from the agricultural fields. Typically washwater contains a disinfectant which, if used correctly, can reduce the populations of both human pathogens and spoilage microorganisms. Care should be taken to ensure the presence of a sufficient amount of sanitizers in the washwater in order to prevent pathogen cross-contamination. Traditionally, chlorine in the form of a sodium hypochlorite solution or as a dry, powdered calcium hypochlorite is used in hydrocooling or washwater as a disinfectant. However, the reaction of chlorine with other organic compounds in perishable produce may lead to the formation of halogenated by-products in the presence of organic matter, giving rise to toxicity concerns. In some European countries, including Germany, the Netherlands, Switzerland, and Belgium, the use of chlorine in RTE products is prohibited (Rico et al., 2007). Moreover, the efficacy of chlorine to reduce microbial pathogens on vegetables is often limited by pH (i.e., hypochlorous acid – the form with the most antimicrobial activity – is present at pH 6.5 to 7.5), temperature, exposure to light, levels of soil and organic matter, initial and residual free chlorine concentration, and length of exposure (Gonzalez et al., 2004; Shen et al., 2013). Several innovative approaches both chemically and physically have been explored for the decontamination of fresh or fresh-cut vegetables (Table 17.5). Effective washing and decontamination of fresh-cut vegetables is difficult to achieve due to the different types of vegetables, the inadequate efficacy of individual treatments alone (Table 17.5), the presence of biofilms on vegetables and on processing equipment (Jahid and Ha, 2012; Somers et al., 1994), and internalization/infiltration of bacteria within produce (Zhuang et al., 1995; Zheng et al., 2013; Takeuchi and Frank, 2000). Therefore, a combination of different disinfection methods (e.g., hurdle technology) (Rico et al., 2007; Joshi et al., 2013) is necessary to increase the efficacy of disinfectants against microbial population reduction. Novel biocontrol strategies using environmentally and ecologically friendly bacterial epiphytes, designed and now being evaluated with human health end points (i.e., prevention of salmonellosis and other produce-borne illnesses), may also play a significant role in the reduction of pathogen loads on fresh produce. Given the lack of effective technologies to eliminate pathogens from produce surfaces and the potential for pathogen cross-contamination during produce washing and post-harvest handling, preventing pathogen proliferation via temperature control is critical to mitigate food safety risks. Overall, technology advances spanning the detection, monitoring, and tracking of food-borne pathogens along with more highly effective preventive control and kill-step measures will be crucial to maintaining a safe fresh and fresh-cut produce supply for consumers in the US and around the world.

References


