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Current focus

Ecological factors influencing survival and growth of human pathogens on raw fruits and vegetables

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Abstract

Outbreaks of human infections associated with consumption of raw fruits and vegetables have occurred with increased frequency during the past decade. Factors contributing to this increase may include changes in agronomic and processing practices, an increase in per capita consumption of raw or minimally processed fruits and vegetables, increased international trade and distribution, and an increase in the number of immuno-compromised consumers. A general lack of efficacy of sanitizers in removing or killing pathogens on raw fruits and vegetables has been attributed, in part, to their inaccessibility to locations within structures and tissues that may harbor pathogens. Understanding the ecology of pathogens and naturally occurring microorganisms is essential before interventions for elimination or control of growth can be devised. © 2002 Éditions scientifiques et médicales Elsevier SAS. All rights reserved.

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1. Introduction

The number of documented outbreaks of human infections associated with consumption of raw fruits, vegetables, and unpasteurized fruit juices has increased in recent years [1–3]. Advances in epidemiologic surveillance programs have enabled these associations to be made. However, changes in dietary habits, methods of fruit and vegetable production and processing, sources of produce, and the emergence of pathogens previously not recognized for their association with raw produce have enhanced the potential for outbreaks [4,5].

While much is known about the ecology of microbial pathogens in foods of animal origin, the behavior of pathogens in association with naturally occurring microflora on fruits and vegetables is ill-defined. Tremendous differences in surface morphology, internal tissue composition, and metabolic activities of leaves, stems, florets, fruits, roots, and tubers provide a wide range of diverse ecological niches selective for specific species or groups of microorganisms.

Bruised and cut surface tissues exude fluids containing nutrients and numerous phytoalexins and other antimicrobials that may enhance or retard the growth of naturally occurring microflora and pathogens [6]. The presence of soil or fecal material on the surface of produce that may permeate cut tissues may alter the ecological environment and, perhaps, also the behavior of pathogens and other microflora. The growth of molds in these environments may result in increased pH, thus enhancing the probability of growth of pathogenic bacteria. Colonization and biofilm development may ensue, resulting in conditions that would protect against death of pathogens or promote growth of spoilage or pathogenic microorganisms. The viability of parasites as affected by extrinsic and intrinsic factors unique to fruits and vegetables is unknown.

2. Pathogens isolated from or associated with fruits and vegetables

Although spoilage bacteria, yeasts, and molds dominate the microflora on raw fruits and vegetables, the occasional presence of pathogenic bacteria, parasites, and viruses capable of causing human infections has also been documented (for reviews see [2,7–12]). All types of produce have potential to harbor pathogens [13] but *Shigella* spp., *Salmonella*, enterotoxigenic and enterohemorrhagic *Es*

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cherichia coli, *Campylobacter* spp., *Listeria monocytogenes*, *Yersinia enterocolitica*, *Bacillus cereus*, *Clostridium botulinum*, viruses, and parasites such as *Giardia lamblia*, *Cyclospora cayetanensis*, and *Cryptosporidium parvum* are of greatest public health concern [2,7,14,15]. Fruits and vegetables can become contaminated with pathogenic microorganisms while growing in fields, orchards, vineyards, or greenhouses, or during harvesting, post-harvest handling, processing, distribution, and preparation in food service or home settings. Listed in Table 1 are examples of fresh vegetables from which pathogenic bacteria have been isolated. Each vegetable in this list possesses a unique set of intrinsic factors that can influence the survival and growth of human pathogenic microorganisms.

Table 1
Examples of pathogenic bacteria isolated from raw vegetables [a]

Vegetable	Country	Pathogen	Prevalence [b]	Reference
Alfalfa sprouts	USA	*Aeromonas*		[16]
	USA	*S.* Meleagridis		[17]
	USA	*Bacillus cereus*		[18]
Alfalfa seeds	USA	*S.* Havana		[19]
		S. Cubana		
		S. Tennessee		
	USA	*S.* Newport		[20]
	Denmark			
Artichoke	Spain	*Salmonella*	3/25 (12%)	[21]
Asparagus	USA	*Aeromonas*		[22]
Bean sprouts	Malaysia	*L. monocytogenes*	6/7 (85%)	[23]
	Sweden	*Salmonella*		[24]
	Thailand	*Salmonella*	30/344 (8.7%)	[25]
Beet leaves	Spain	*Salmonella*	4/52 (7.7%)	[21]
Broccoli	USA	*Aeromonas*		[26]
	USA	*Aeromonas*	5/16 (31.3%)	[16]
Cabbage	Canada	*L. monocytogenes*	2/92 (2.2%)	[27]
	Mexico	*E. coli* O157:H7	1/4 (25.0%)	[28]
	Peru	*Vibrio cholerae*		[29]
	Spain	*Salmonella*	7/41 (17.1%)	[21]
	Sri Lanka	*L. monocytogenes*	6/18 (33%)	[30]
	USA	*L. monocytogenes*	1/92 (1.1%)	[31]
Carrots	Lebanon	*Staphylococcus*	(14.3%)	[32]
Cauliflower	Netherlands	*Salmonella*	1/13 (7.7%)	[33]
		Salmonella	1/23 (4.5%)	[21]
Celery	USA	*Aeromonas*		[26]
	Mexico	*E. coli* O157:H7	6/34 (17.6%)	[28]
Chili	Spain	*Salmonella*	2/26 (7.7%)	[21]
Cilantro	Surinam	*Salmonella*	5/16 (31.3%)	[33]
Coriander	Mexico	*E. coli* O157:H7	8/41 (19.5%)	[28]
Cress sprouts	Mexico	*E. coli* O157:H7	2/20 (20.0%)	[28]
Cucumber	USA	*B. cereus*		[18]
	Malaysia	*L. monocytogenes*	4/5 (80%)	[23]
	Pakistan	*L. monocytogenes*	1/5 (6.7%)	[34]
Egg plant	USA	*L. monocytogenes*	2/92 (2.2%)	[31]
Endive	Netherlands	*Salmonella*	2/13 (1.5%)	[33]
Fennel	Netherlands	*Salmonella*	2/26 (7.7%)	[33]
Green onion	Italy	*Salmonella*	4/89 (71.9%)	[35]
Leafy vegetables	Canada	*Campylobacter*	1/40 (2.5%)	[36]
Lettuce	Malaysia	*L. monocytogenes*	5/22 (22.7%)	[23]
	Canada	*Campylobacter*	2/67 (3.1%)	[36]
	Italy	*Salmonella*	82/120 (68.3%)	[35]
	Lebanon	*Staphylococcus*	(14.3%)	[32]
	Malaysia	*L. monocytogenes*	1/28 (3.6%)	[37]
	Netherlands	*Salmonella*	2/28 (7.1%)	[33]
	Spain	*Salmonella*	5/80 (6.3%)	[21]
	Sri Lanka	*L. monocytogenes*	10/20 (50%)	[30]
	USA	*Aeromonas*		[16]
Mungbean sprouts	UK	*S.* Saint-Paul		[38]
Mushrooms	US	*C. jejuni*	3/200 (1.5%)	[39]
Mustard cress	UK	*S.* Gold-Coast		[40]
Mustard sprouts	USA	*B. cereus*		[18]
Parsley	Canada	*Campylobacter*	1/42 (2.4%)	[36]
	Egypt	*Shigella*	1/250 (0.4%)	[41]
	Lebanon	*Staphylococcus*	(7.7%)	[32]
	Spain	*Salmonella*	1/23 (4.3%)	[21]

Vegetable	Country	Pathogen	Prevalence [b]	Reference
Pepper	Canada	*Campylobacter*	1/63 (1.6%)	[36]
	USA	*Aeromonas*		[16]
	Sweden	*Salmonella*		[42]
Potatoes	USA	*L. monoyctogenes*	19/70 (27.1%)	[31]
	USA	*L. monocytogenes*	28/132 (21.2%)	[43]
Prepacked salads	Canada	*Campylobacter*	2/74 (2.7%)	[36]
	Northern Ireland	*L. monocytogenes*	3/21 (14.3%)	[44]
	UK	*L. monocytogenes*	4/60 (13.3%)	[45]
Radish	Lebanon	*Staphylococcus*	(6.3%)	[32]
	USA	*L. monocytogenes*	25/68 (36.8%)	[31]
	USA	*L. monocytogenes*	19/132 (14.4%)	[43]
Salad greens	Egypt	*Salmonella*	1/250 (0.4%)	[41]
	UK	*S. aureus*	13/256 (5.1%)	[46]
Salad vegetables	Egypt	*Shigella*	3/250 (1.2%)	[41]
	Egypt	*S. aureus*	3/36 (8.3%)	[32]
	Germany	*L. monocytogenes*	6/263 (2.3%)	[47]
	Northern Ireland	*L. monocytogenes*	4/16 (25%)	[44]
	UK	*Y. enterocolitica*	4/16 (25%)	[48]
	UK	*L. monocytogenes*	2/108 (1.8%)	[49]
Seed sprouts	Canada	*Staphylococcus*	13/45 (24%)	[50]
Soybean sprouts	USA	*B. cereus*		[18]
Spinach	Canada	*Campylobacter*	2/60 (3.3%)	[36]
	Spain	*Salmonella*	2/38 (5.2%)	[21]
	USA	*Aeromonas*		[16]
Sprouting seeds	USA	*B. cereus*	56/98 (57%)	[51]
Tomato	Pakistan	*L. monocytogenes*	2/15 (13.3%)	[34]
Vegetables	Egypt	*Salmonella*	2/250 (0.8%)	[41]
	France	*Y. enterocolitica*	4/58 (7%)	[52]
	France	*Y. enterocolitica*	15/30 (50%)	[53]
	Iraq	*Salmonella*	3/43 (7.0%)	[54]
	Italy	*L. monocytogenes*	7/102 (6.9%)	
	Italy	*Y. enterocolitica*	1/102 (1.0%)	[55]
	Spain	*L. monocytogenes*	8/103 (7.8%)	[56]
	Spain	*Salmonella*	46/849 (5.4%)	[57]
	Taiwan	*L. monocytogenes*	6/49 (12.2%)	[58]
	UK	*L. monocytogenes*	4/64 (6.2%)	[59]
	USA	*Salmonella*	4/50 (8.0%)	[60]

[a] Adapted from [7] and used with permission of the International Association of Food Protection.

[b] Number of samples positive out of number analyzed; percentage of positive samples is in parenthesis.

A wide range of fresh fruits and vegetables, as well as unpasteurized fruit juices, has been implicated in outbreaks of infections. Examples are listed in Table 2. This is not a comprehensive list but does illustrate the diversity of types of produce potentially capable of serving as vehicles for human infection. The survival and growth of a pathogen on or in raw produce or unpasteurized produce products are dictated by its metabolic capabilities. 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 the entire system of production, processing, distribution, and preparation at the site of consumption.

3. Possible reasons for increased numbers of outbreaks or infections

Changes in agronomic, processing, preservation, packaging, distribution, and marketing technologies on a global scale have enabled the raw fruit and vegetable industry to supply consumers with a wide range of high-quality produce in most countries year round. Some of the same technologies and practices have also introduced an increased risk for human illness associated with pathogenic microorganisms. The use of animal manure that has not been composted rather than chemical fertilizer, as well as untreated sewage or irrigation water containing pathogens contributes to this risk. Changes in social demographics, food consumption patterns, and awareness by epidemiologists and health care professionals that raw fruits and vegetables are potential vehicles of infections may also be contributing to an increase in documented produce-associated outbreaks of human illness.

Many of the factors that are thought to contribute to the epidemiology of diseases associated with raw fruits and vegetables [5,110] are directly or indirectly impacted by ecological conditions that affect survival or growth of pathogenic microorganisms. Adaptation to stress environments can result in a pathogen becoming better suited to survival and growth, or to becoming more virulent. *E. coli*

Table 2
Examples of outbreaks of infections epidemiologically associated with raw fruits and vegetables and unpasteurized products

Microorganism	Year	Location	Type of produce or product	Reference
Bacteria				
Bacillus cereus	1973	USA	Seed sprouts	[61]
Clostridium botulinum	1987	USA	Cabbage	[62]
E. coli O157:H7	1991	USA	Apple cider	[63]
	1995	USA	Lettuce	[64,65]
	1996	USA	Apple juice	[66,67]
	1997	Japan	Radish sprouts	[68]
	1997	USA	Alfalfa sprouts	[69]
E. coli (enterotoxigenic)	1993	USA	Carrots	[70]
Listeria monocytogenes	1979	USA	Celery, lettuce, tomato	[71]
	1981	Canada	Cabbage	[72]
Salmonella				
Miami	1954	USA	Watermelon	[73]
Typhimurium	1974	USA	Apple cider	[3]
Oranienburg	1979	USA	Watermelon	[74]
Saint-Paul	1988	UK	Mungbean sprouts	[75]
Chester	1989-90	USA	Cantaloupes	[76]
Javiana	1990	USA	Tomatoes	[77]
Poona	1991	USA/Canada	Cantaloupes	[78]
Montevideo	1993	USA	Tomatoes	[5]
Bovismorbificans	1994	Sweden/Finland	Alfalfa sprouts	[79]
Hartford/Gaminara/Rubislaw	1995	USA	Orange juice	[80]
Stanley	1995	USA	Alfalfa sprouts	[81]
Montevideo/Meleagridis	1996	USA	Alfalfa sprouts	[17]
Typhi	1998-99	USA	Mamey	[82]
Mbandaka	1999	USA	Alfalfa sprouts	[83]
Shigella flexneri	1998	UK	Fruit salad	[84]
S. sonnei	1986	USA	Lettuce	[85]
	1994	Norway	Lettuce	[86]
	1998	USA	Parsley	[87]
	1995	USA	Scallions	[88]
Vibrio cholerae	1970	Israel	Vegetables	[89]
	1991	USA	Coconut milk	[90]
Viruses				
Calicivirus	1998	Finland	Raspberries (frozen)	[91]
Hepatitis A	1983	UK	Raspberries (frozen)	[92]
	1988	USA	Lettuce	[93]
	1990	USA	Strawberries (frozen)	[94]
	1994	USA	Tomatoes	[95]
	1997	USA	Strawberries	[96]
Norwalk and Norwalk-like	1987	UK	Melon	[97]
	1982	USA	Green salad	[98]
	1991	USA	Celery	[99]
Parasites				
Cyclospora cayetanensis	1996-97	USA, Canada	Raspberries	[100–104]
	1997	USA	Lettuce	[105]
	1997	USA	Basil/basil-containing products	[106]
	1997	Peru	Raw vegetables	[14]
Cryptosporidium parvum	1993	USA	Apple cider	[107]
	1995	USA	Mixed salad with celery	[108]
	1996	USA	Apple cider	[67]
	1997	Peru	Raw vegetables	[14]
Giardia lamblia	1992	USA	Raw vegetables	[109]

O157:H7 [111–113] and *Salmonella* [114,115], for example, are known to adapt to reduced pH and subsequently exhibit increased tolerance to stress environments. Global trade and international travel have resulted in increased contact of people with pathogens to which they had not been previously exposed. Trends toward greater geographic distribution of minimally processed fruits and vegetables from central processing facilities and subsequent storage and handling practices in food preparation areas may also be contributing to an increased frequency of produce-associated infections. The ecological behavior of pathogens and spoilage microorganisms on raw fruits and vegetables can be greatly affected by these changes, thus resulting in increased risk of illness.

4. Understanding the ecosystem of pathogens is paramount to devising methods for control

A better understanding of microbial ecosystems on the surface of raw fruits and 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 production, processing, marketing, and preparation for consumption. These ecosystems are extremely diverse and complex. The presence and numbers of bacteria, yeasts, molds, parasites, and viruses differ, depending on the type of produce, agronomic practices, geographical area of production, and weather conditions prior to harvest [13,116,117]. Microbial ecosystems unique to various types of produce after harvesting can be greatly influenced by handling and storage conditions as well as conditions of processing, packaging, distribution, and marketing.

Pathogens, along with spoilage microorganisms, may contaminate fruits and vegetables via several different routes and at several points throughout the pre- and post-harvest system. Potential pre-harvest sources of microorganisms include soil, feces, irrigation water, water used to apply fungicides and insecticides, dust, insects, inadequately composted manure, wild and domestic animals, and human handling. Post-harvest sources include feces, human handling, harvesting equipment, transport containers, wild and domestic animals, insects, dust, rinse water, ice, transport vehicles, and processing equipment. Janisiewicz et al. [118] demonstrated that fruit flies contaminated with a fluorescent-tagged nonpathogenic strain of *E. coli* O157:H7 served as a vector in colonization of the organism in apple wounds. The bacterium was isolated from apple wounds within 48 h of exposure of apples to the flies.

Manure used as a fertilizer or soil amendment, as well as in irrigation water, represents a potential source of pathogens to contaminate fruits and vegetables. *E. coli* O157:H7 and *Salmonella* are carried by animals and shed in their feces [119,120]. Noncomposted or improperly composted manure used on the farm, or manure that enters surface waters, may contain these pathogens and subsequently contaminate produce [121]. Studies on the fate of *E. coli* O157:H7 in bovine feces revealed that the pathogen survived in cattle manure for 42–49 d at 37 °C, and for 49–56 d at 22 °C [122]. Another study on the behavior of *E. coli* O157:H7 in manure revealed that the pathogen could survive up to 47 d, 4 months, and 21 months, in bovine manure, aerated ovine manure, and nonaerated ovine manure, respectively [123]. Beuchat [124] was able to detect *E. coli* O157:H7 on manure-contaminated lettuce stored at 4 °C for up to 15 d, even when the initial inoculum was only 10^0-10^1 CFU/g.

L. monocytogenes is widely distributed in the environment, where it is associated with decaying vegetation, soil, sewage, and feces of animals, and has been isolated from several types of vegetables [7]. Cases of human listeriosis that have been associated with the consumption of raw vegetables are likely, in part, due to contamination by manure from ruminants [8]. *L. monocytogenes* is known to grow on a variety of vegetables at refrigeration temperatures [13,125,126].

Although produce may become contaminated with pathogens as a result of contact with manure used as a soil fertilizer, through manure-contaminated irrigation water, or by direct contact with feces from birds and grazing animals, very little is known regarding the effect of ecological conditions on survival of pathogens enmeshed in manure on the surface of fruits and vegetables. Likewise, although the use of manure in the production of fruits and vegetables should be carefully managed to limit the potential for contamination with pathogens during pre-harvest, there are few scientific data to validate practical treatments to kill pathogens in manure that may adhere to fresh produce.

Intrinsic as well as extrinsic factors determine the range and populations of microorganisms associated with fruits and vegetables at any given point throughout their production and post-harvest handling, thus influencing the rate and type of spoilage that eventually renders raw produce inedible. Surfaces of fruits, stems, roots, florets, and leaves, for example, are each characterized by unique microenvironments that influence colonization of bacteria, yeasts, and molds, as well as attachment of these microorganisms, parasites, and viruses. The environment in which plants are grown imposes extrinsic factors that influence survival and growth of associated surface microflora, whereas intrinsic parameters such as the nature of the epithelium and protective cuticle, tissue pH, and the presence of antimicrobials dictate which groups of produce may be more likely than others to harbor certain types of microorganisms in damaged tissues.

The range of microorganisms recovered from raw fruits and vegetables at harvest most often reflects the microflora present in the field, orchard, grove, or vineyard at the time of harvest [2,117,127]. Climatic and agricultural determinants affecting the microbial ecosystem at harvest include geographical location, history of precipitation, wind, irrigation practices, pre-harvest, harvest, and post-harvest practices, and the presence of insects, animals, and birds [4,13]. Gram-negative bacteria dominate the microflora associated with most vegetables, while molds and weakly fermentative yeasts often comprise the majority of microflora on raw fruits, largely due to the acidic pH of fruit tissue, which generally is less than 4.0 [128].

5. Cohabitation with other microorganisms may affect survival and growth of pathogens

While the pH of many vegetables is in a range suitable for growth of pathogenic bacteria, some, e.g. fully ripe tomatoes, are in a pH range (3.9–4.4) that prevents or retards growth. Yeasts and molds, on the other hand, have a competitive advantage over bacteria that may access bruised tissues of acidic vegetables and many fruits, because they are able to grow at the lower pH range (2.2–5.0) characteristic of much of this produce. Spoilage of fruits is often caused by specific molds or groups of molds and yeasts [128]. When surface tissues of fruits are punctured or broken by insects or mechanical abuse, yeasts and molds naturally present on the skin surface can rapidly grow in the abundance of nutrients available in the released cell fluids.

Spoilage by yeasts usually results from fermentative activity. Molds, many of which can utilize ethanol and simple sugars as sources of energy, then grow and eventually degrade structural polysaccharides. Many molds produce ammonia and other alkaline by-products during the course of metabolizing substrate nutrients. Some molds and yeasts utilize organic acids, leading to reduced acidity and increased pH. At least two reports show that growth of molds on the surface of tomato juice (pH 4.2) increases the pH to 6.3–7.6, allowing *C. botulinum* to grow and produce toxin [129,130]. Of the 58 species representing 21 genera of molds examined by Mundt [131], all except two raised the pH of tomato juice (pH 4.1) to a range of 4.9–9.0.

Insects such as the lesser mealworm and house fly have been shown to carry *E. coli* O157:H7 [132,133]. Fruits flies have been shown to transmit *E. coli* O157:H7 to apples [118]. Recognizing that outbreaks of *E. coli* O157:H7 infections associated with apple cider may have been due, in part, to amplification of the pathogen within bruised apple tissue, Dingman [134] investigated survival and growth of *E. coli* O157:H7 in tissue of five apple cultivars. While growth occurred in bruised tissue of all five cultivars, initiation of growth varied from 2 to 6 d after inoculation and was influenced by the time elapsed between picking and inoculating apples. Bacteria other than *E. coli* O157:H7 were not isolated from inoculated bruised tissue. Yeast and mold populations were not determined; however, the pH of bruised apples was significantly higher than the pH of undamaged apples, suggesting that mold growth may have occurred. In any case, the increased pH would favor survival and growth of *E. coli* O157:H7 and other pathogens.

In a survey of 401 samples of raw fruits and vegetables collected in retail markets, 66% affected by bacterial soft rot were positive for presumptive colonies of *Salmonella* [135]. Thirty percent of 166 representative isolates from 20 different commodities, including cantaloupe and tomato, were confirmed to be *Salmonella*. Co-inoculation of potato, carrot, and pepper with a soft-rot bacterium and with *Salmonella typhimurium*, followed by incubation for 24 h, resulted in 10-fold higher counts of the pathogen compared to vegetables inoculated with *Salmonella* alone. Vegetables co-inoculated with *Pseudomonas viridiflava* and *S. typhimurium* contained *Salmonella* populations approximately three times higher than vegetables inoculated with *Salmonella* alone. Janisiewicz et al. [136], on the other hand, reported that inoculation of *Pseudomonas syringae* into wounds of apples prevented *E. coli* O157:H7 from growing. Populations of yeasts and molds on test produce were not reported in these studies [135,136].

With the exception of some types of melons, e.g. cantaloupe (pH 6.2–6.9) and watermelon (pH 5.2–5.7), which are recognized as good substrates for growth of *Salmonella* [137] and *E. coli* O157:H7 [138], fruits and fruit juices with pH less than 4.0 are generally not considered as substrates to support the growth of pathogenic bacteria. However, the development of a pH gradient surrounding mycelial growth in bruised tissues or as a mat on the surface of juice could provide conditions for growth of incident cells of pathogenic bacteria.

Most of the natural microflora on the surface of fresh produce do not exert a deleterious effect on sensory qualities. However, when spoilage does occur, *Pseudomonas*, *Xanthomonas*, *Erwinia*, *Bacillus*, *Clostridium*, and several genera of yeasts and molds are commonly involved [139,140]. Some naturally occurring microorganisms may have a lethal or antagonistic effect on bacteria capable of causing human diseases. Bacteriocinogenic strains of *Pediococcus* and *Enterococcus*, for example, have recently been shown to control the growth of *L. monocytogenes* on mungbean sprouts [141].

6. Behavior of pathogens in biofilms

Means by which pathogens contaminate fresh produce are several, including environmental sources in the field or orchard, as noted above, or contact with harvesting equipment and containers used to transport produce from the field to the marketplace, and perhaps in food service and home settings. Exopolysaccarides secreted by bacteria can form a bound capsule layer when associated with the cell wall or released by the cell to create a matrix structure [142]. Microbial aggregates that may harbor bacteria, yeasts, and molds within this matrix have been observed on plant surfaces, and these structures are referred to as biofilms [143].

Colonization of spoilage and non-spoilage microorganisms of fruits, vegetables, and post-harvest contact surfaces can provide a protective environment for pathogens, reducing the effectiveness of sanitizers and other inhibitory agents [144]. *L. monocytogenes*, in a multispecies biofilm containing *Pseudomonas fragi* and *Staphylococcus xylosus*, has been reported to be essentially unaffected by treatment with 500 ppm free chlorine [145]. Fett [146] examined the cotyledons, hypocotyls, and roots of alfalfa, broccoli, cloves, and sunflower sprouts. Biofilms were observed on plant parts. He concluded that naturally occurring biofilms on sprouts may afford protected colonization sites for human pathogens such as *Salmonella* and *E. coli* O157:H7, making their elimination with antimicrobial compounds difficult. The formation of biofilms on leaf surfaces of spinach, lettuce, Chinese cabbage, celery, leek, basil, pars ley and endive has been demonstrated [143]. Estimates of biofilm abundance in phyllosphere communities show that bacteria in biofilms constitute 10–40% of the bacterial population on broad-leaf endive and parsley [147].

Containers used to harvest, transport, and display raw fruits and vegetables are often not effectively cleaned and sanitized, which can lead to the development of biofilms [148–150]. Even single-use containers may hold produce for a sufficient time to allow the formation of biofilms. Contamination of fresh produce with pathogens may result from contact with surfaces harboring these biofilms. If pathogens attach to biofilms during transport or processing, their survival and growth may be enhanced [151–153]. Growth of pathogens incorporated into biofilms would increase the probability of cross-contamination of produce. Jeong and Frank [154,155] determined that *L. monocytogenes* grows in multispecies biofilms containing microflora from meat and dairy plants. No information is available on the behavior of *L. monocytogenes* or other pathogenic bacteria in biofilms formed by microflora associated with raw fruits and vegetables. Predominant microorganisms in biofilms on surfaces of containers and equipment used in the fresh fruit and vegetable industry would likely differ greatly from those on containers and equipment used in meat and dairy industries. Even in the produce industry, microflora in biofilms on various container and equipment surfaces would be predicted to differ greatly, depending on the type of produce being harvested or processed. Survival and growth characteristics of pathogens would also likely be influenced by these differences.

During growth and maturation of fruits and vegetables as well as during harvesting, transport, processing, and storage after processing, opportunities arise for the development of biofilms. These biofilms may provide protection against sanitizers. Growth of *L. monocytogenes* in a multispecies biofilm, with concurrent development of resistance to sodium hypochlorite, has been demonstrated [145]. A model system needs to be developed to simulate produce biofilms for the purpose of determining the behavior of pathogens incorporated into them. The ability of pathogens to survive in biofilms subjected to dehydration and treatment with sanitizers needs to be determined.

7. Processing may exacerbate the problem of killing or removing pathogens

The lack of efficacy of sanitizers used to decontaminate the surface of raw fruits and vegetables has been largely attributed to the inability of active components in treatment solutions to reach the site of microbial cells [9]. Infiltration of pathogens into cracks, crevices, and intercellular spaces of fruits and vegetables has been demonstrated by several researchers. Infiltration of tomatoes with *Salmonella* [156–158] and *Erwinia carotovora* [159] and of lettuce [160,161], apples [162,163], and oranges [164] with *E. coli* O157:H7 has been described. Once positioned in the these ecological niches, cells may survive and grow to high population by the time the infected produce is consumed.

Infiltration of pathogens into fruit and vegetable tissues is dependent upon temperature, time, and pressure, and only occurs when the water pressure on the produce surface overcomes internal gas pressure and the hydrophobic nature of the produce surface [158,159,163]. Infiltration is enhanced if the temperature of the fruit or vegetable is higher than the temperature of a water suspension of cells. Addition of detergents (surfactants) to water also promotes infiltration of produce, apparently by reducing the surface tension of the water at the air–water interface with damaged cutin, parenchemal cells, or pores leading into the tissues. Regardless of the mode of infiltration, cells may establish microcolonies that are extremely difficult to reach with aqueous chemical solutions. This can result in a higher risk of consuming raw fruits and vegetables that may harbor pathogenic microorganisms.

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