Fitness of Human Enteric Pathogens on Plants and Implications for Food Safety[1]

Maria T. Brandl

Produce Safety and Microbiology Research Unit, Agricultural Research Services, U.S. Department of Agriculture, Albany, California 94710; email: mbrandl@pw.usda.gov

Annu. Rev. Phytopathol.
2006. 44:367–92

First published online as a Review in Advance on April 21, 2006

The *Annual Review of Phytopathology* is online at phyto.annualreviews.org

doi: 10.1146/annurev.phyto.44.070505.143359

0066-4286/06/0908-0367$20.00

Key Words

human pathogen, produce, fitness, foodborne illness, outbreaks

Abstract

The continuous rise in the number of outbreaks of foodborne illness linked to fresh fruit and vegetables challenges the notion that enteric pathogens are defined mostly by their ability to colonize the intestinal habitat. This review describes the epidemiology of produce-associated outbreaks of foodborne disease and presents recently acquired knowledge about the behavior of enteric pathogens on plants, with an emphasis on *Salmonella enterica*, *Escherichia coli* O157:H7, and *Listeria monocytogenes*. The growth and survival of enteric pathogens on plants are discussed in the light of knowledge and concepts in plant microbial ecology, including epiphytic fitness, the physicochemical nature of plant surfaces, biofilm formation, and microbe-microbe and plant-microbe interactions. Information regarding the various stresses that affect the survival of enteric pathogens and the molecular events that underlie their interactions in the plant environment provides a good foundation for assessing their role in the infectious dose of the pathogens when contaminated fresh produce is the vehicle of illness.

Outbreak: the sudden increase in the incidence of a disease above what would be expected at a given time and geographic scale

Enteric pathogens: microbes that occur pathogenically in the intestine of human or animals

Phyllosphere: the living space of the leaf surface

Rhizosphere: the living space of the root surface and the soil that is loosely attached to it

Epiphytic: state by which microbes colonize the surfaces of plants without causing disease

Zoonotic: a disease that can be transmitted from animals to humans

A study has been made of contaminated vegetables as one of the factors in transmitting typhoid infection, with additional reference to the longevity of *Bacillus typhosus* in polluted soil.

R.H. Creel, 1912

INTRODUCTION

The emergence of outbreaks of foodborne illness associated with fresh fruits and vegetables has revived interest among public health agencies and sparked a new wave of research on food safety issues related to microbial contamination of fresh produce. Whereas scientists such as Creel in the early 1900s (28) were attempting to find evidence that typhoid infection spread from contaminated water and food, and thus to disprove the hypothesis that *Bacillus typhosus* (*Salmonella enterica* serovar Typhi) was an obligate parasite, microbiologists today face the challenge of providing scientific evidence for the fitness of enteric pathogens on plants and its link to the rise of foodborne illness incidence from produce. An emphasis on the ecology of enteric pathogens on plant surfaces has placed this field of study at the crossroads of research in food safety, medical microbiology, and plant microbial ecology.

Despite a wealth of information on the interaction of enteric pathogens with their human and animal hosts, acquisition of fundamental knowledge about their behavior on plants has just begun. With the belief that most illness linked to fresh produce was the result of postharvest microbial contamination rather than contamination in the field, early research relied on traditional methods of food microbiology using harvested produce or cut plant tissue to study the biology of enteric pathogens on plants. However, with the recognition of preharvest crop contamination, new ecological questions regarding the fitness of enteric pathogens on crop plants need to be addressed. Plant pathology, including its subdisciplines of phyllosphere and rhizosphere microbiology, has helped to develop scientific methodologies and key concepts in microbial ecology to address practical problems in plant disease management. These concepts, such as bacterial epiphytic fitness, endophytic growth and survival, resource utilization in the phyllosphere and the rhizosphere, plant-microbe interactions, and microbe-microbe interactions, provide a platform to formulate hypotheses on the ecology of enteric pathogens on plants. This article presents an overview of issues related to the microbial contamination of fresh produce with a focus on the behavior of enteric pathogens on plant surfaces in the context of concepts in food safety and plant microbiology.

AGRICULTURAL PLANTS AS VEHICLES OF FOODBORNE ILLNESS

A New Challenge in Food Safety

The epidemiology of foodborne disease has changed rapidly over the past two decades. Shortly after some major human pathogens were recognized as being spread from animal reservoirs, fresh fruits and vegetables emerged as new vehicles for the transmission of these zoonotic diseases (149). The occurrence of foodborne illness from contaminated fresh produce challenged the belief that such disease was linked to the consumption of foods of animal origin, including meat, poultry, eggs, and milk. Early epidemiological investigations of produce as a source of infection were triggered mostly by the increased isolation of a rare species or serovar of enteric pathogens from clinical patients; thus, outbreaks from common types of pathogens may have remained undetected (149). Since the early 1990s, awareness of the potential of fresh produce to cause foodborne disease has increased, and reported outbreaks associated with this commodity have grown steadily (135). The factors that have likely contributed

Table 1 Factors involved in the emergence of produce-linked outbreaks[a]

Changes in the produce industry
Intensification and centralization of production
Wider distribution of produce over longer distances
Introduction of minimally processed produce
Increased importation of fresh produce
Changes in consumer habits
Increased consumption of meals outside the home
Increased popularity of salad bars
Increased consumption of fresh fruits and vegetables, and fresh fruit juices
Increased size of at-risk population (elderly, immunocompromised)
Enhanced epidemiological surveillance
Improved methods to identify and track pathogens
Emerging pathogens with low infectious dose

[a]Derived from Tauxe et al. (148).

to the emergence of produce as a source of enteric illness are listed in **Table 1**. New scientific evidence for the growth and survival of enteric pathogens on fruit and vegetables (see below) has provided a biological basis to further investigate these commodities as a link to enteric disease.

Sources of Contamination

In addition to the major factors in the emergence of produce-associated outbreaks listed in **Table 1**, intensification of animal production raises the specter that the sources of contamination in the environment are themselves increasing. **Figure 1** presents a schematic diagram of the various sources of contamination that may introduce human pathogens into the field and contribute to the contamination cycle in agricultural areas. Manure is commonly applied to fields in order to dispose of animal waste and to fertilize soils. Enteric pathogens can survive for prolonged periods of time in animal feces (53, 58, 77) and may serve as potential inoculum onto plants in the field. Several studies have demonstrated the presence of foodborne pathogenic bacteria on crops grown in soil to which naturally or artificially contaminated manure was applied (136). Thus, the use of improperly composted manure or the feces from free roaming domestic or wild animals in the fields, enhance the risk of microbial contamination of crops. Additionally, poor hygiene practices by field-workers and a lack of on-site sanitation facilities may result in produce-associated outbreaks, particularly enteric illness such as shigellosis, which is easily contracted from human feces because of the low infectious dose of the causal agent, *Shigella* (102).

Infectious dose: the number of infecting organisms needed to cause disease

Crop irrigation and application of pesticides with contaminated water also are considered as primary sources of inoculum in the field. This is of particular concern for production of fruits and vegetables in areas where the supply of fresh water is scarce, and where water reclaimed from effluents increasingly serves for agricultural purposes. Because *E. coli* and *S. enterica* survive well in water sediments (45, 51), seasonal flooding of fields with overflowing stream water should be added to the risk factors of potential crop contamination.

Whether airborne transmission of enteric pathogens has a role in their contamination of plants in the field is not known. In a study of bacterial migration in orange orchards, the aerial dispersal of epiphytic bacteria from source plants was demonstrated to be an important factor in the composition of leaf microbial communities in neighboring trees, particularly when the plant surface onto which the bacteria immigrated was unfavorable for bacterial growth (86). Although bioaerosols have long been recognized

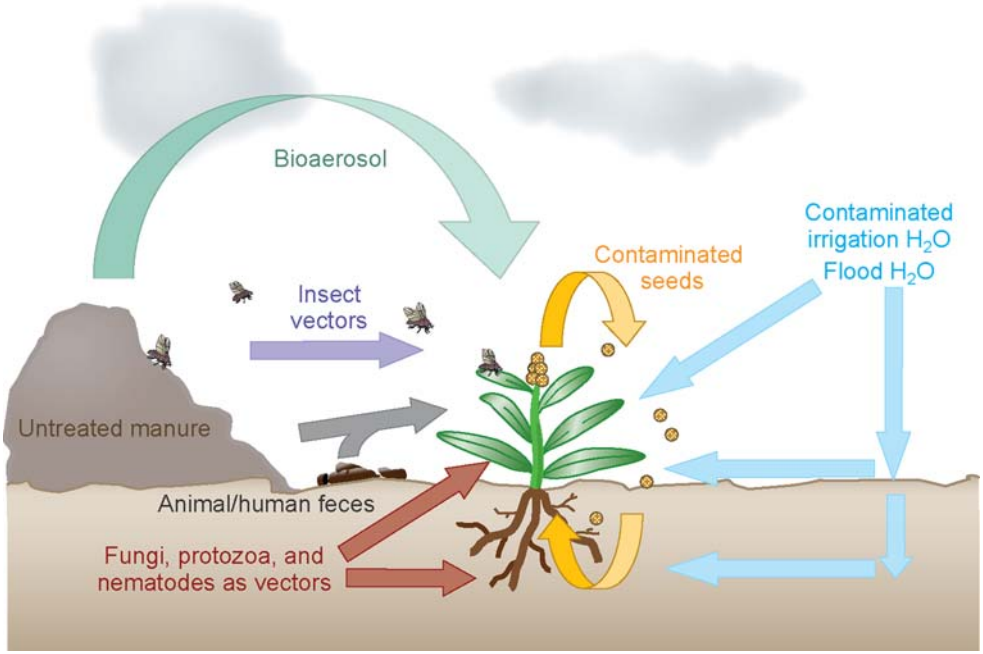

Figure 1

Schematic illustration of factors that can contribute to the contamination of fruit and vegetables with human enteric pathogens in the field.

Etiologic agent: organism that causes a disease

as the primary mode of pathogen spread among livestock (37), the potential for air currents to disperse enteric pathogens from manure piles or contaminated soil, and to enable their immigration as viable cells onto crops, remains to be explored.

Evidence was found for the transmission of pathogenic *E. coli* via insect vectors such as the fruit fly (*Ceratitis capitata*) (70) and the vinegar fly (*Drosophila melanogaster*) (134) to apples, and for its excretion from inoculated houseflies (130). Plant pathogenic bacteria and their antagonists are effectively vectored by honeybees on and among apple and pear flowers in fruit orchards (73), and the dissemination of epiphytic bacteria by insects on wet leaf surfaces has been reported (56). The ubiquitous presence of insects on manure piles, in feedlots, and also in fruit and vegetable fields warrants examination of this type of transmission as a factor in the preharvest contamination of produce.

Outbreaks of Foodborne Illness and Incidence of Enteric Pathogens on Plants

Epidemics of foodborne illness linked to fresh produce increased in the United States from 1973 through 1997, both in absolute number and in the proportion of total food-linked outbreaks with a known etiologic agent (135). A recent survey revealed that produce caused the most cases (28,315 cases) of foodborne disease, and was the second most common single-food vehicle linked to outbreaks in the United States in the period of 1990–2003 (22). In other countries where surveillance of foodborne illness is extensive, a significant proportion of outbreaks has also been attributed to fresh produce. For example, in England and Wales, salad, vegetables, and fruit caused 6.4% and 10.1% of all outbreaks with a known food vehicle in the periods of 1993–1998 (173) and 1999–2000 (174), respectively. Lettuce, melon, seed sprouts, and fruit juice were the

four most important single produce items implicated in epidemics of foodborne illness in the United States between 1973 and 1997 (135). Other fresh produce associated with outbreaks of bacterial enteric disease include tomato (30, 50, 156, 157), cilantro (17, 151), parsley (107), spinach (157), green onions (148), carrot (155), and cabbage (133).

Early outbreaks of foodborne disease epidemiologically linked to fresh produce were initially suspected to result from cross-contamination of fruit or vegetables during food preparation with food items more likely to be a source of enteric illness, particularly meat products. However, recent large surveys conducted by the U.S. Food and Drug Administration with samples obtained from major distributors indicated that 1.6% (162) and 4.4% (161) of the domestic and imported produce, respectively, were contaminated with human pathogens. Another surveillance study by the Microbial Data Program of the U.S. Department of Agriculture reported that in 2002, 0.62% of samples of domestic or imported cantaloupe, celery, lettuce, and tomatoes were contaminated with *E. coli* strains harboring virulence factors (160). Several smaller surveys have demonstrated the presence of pathogenic enteric bacteria on produce and in unpasteurized fruit or vegetable juices sampled during production or at retail markets (48). Therefore, surveillance data support the hypotheses of possible contamination of fresh produce with human pathogens in the preharvest environment, or in a postharvest fashion during washing and minimal processing, and consequently, of outbreaks linked to fresh produce not necessarily being the outcome of cross-contamination events with other foods.

Several bacterial pathogens have caused fresh produce-associated epidemics of enteric illness, including *Salmonella enterica*, pathogenic *E. coli*, *Shigella* spp., *Campylobacter* spp., *Listeria monocytogenes*, *Staphylococcus aureus*, *Yersinia* spp., and *Bacillus cereus*. *S. enterica*, the most frequent etiologic agent of outbreaks from fresh produce, caused 48% of such outbreaks with a known etiology between 1973 and 1997 in the United States (135). Of particular concern is the occurrence of outbreaks caused by multidrug-resistant strains of *S. enterica* serovar Typhimurium DT104 and linked to the consumption of lettuce (57, 147). Pathogenic *E. coli* is the second most important causal agent of outbreaks from fresh produce (135). Pathogenic strains of *E. coli* include the enterotoxigenic serotype O157:H7, a dangerous foodborne pathogen that can cause hemolytic uremic syndrome and lead to death, particularly in children, the elderly, and the immunocompromised. *E. coli* O157:H7 caused 21% of all produce-linked outbreaks in 1982–2002 in the United States (121).

Enteric pathogens other than *S. enterica* and *E. coli* have been implicated in relatively few produce-associated outbreaks. This is also illustrated in **Table 2**, which lists the number of outbreaks linked to a single fresh produce item in the United States between 1990 and 2004. These data were categorized by causal agent and by plant part or entity that the produce represents; mixed salads, which are associated with a high incidence of foodborne illness, are not included. Not listed are root vegetables, which have rarely been implicated in outbreaks, possibly because they are commonly cooked before consumption. **Table 2** reveals interesting trends in the type of plant part linked to illness by *S. enterica* and pathogenic *E. coli*. For example, during this 14-year period, 76% of the epidemics from contaminated fruit were caused by *S. enterica*, whereas pathogenic *E. coli* strains were the causal agent of the highest proportion (48%) of foodborne illness outbreaks

HISTORICAL EPIDEMIC LINKED TO PRODUCE

E. coli O157:H7 is the causal agent of the largest outbreak of bacterial enteric disease in recent times, with over 6000 cases epidemiologically linked to contaminated radish sprouts in Japan in 1996 (108, 167).

Epidemiologically linked: epidemiological data link the disease to the consumption of a (or multiple) food(s). The short shelf life of fresh produce makes it difficult to isolate the causative agent from this type of food, and epidemiological studies often are used to trace back the source of contamination

Table 2 Number of outbreaks[a] linked to single items of fresh produce in the United States in 1990–2004

Produce type	*S. enterica*	Pathogenic *E. coli*	*Shigella* spp.	*Campylobacter* spp.
Fruit[b]	32 (76)[c]	8 (19)	1 (2)	1 (2)
Leafy vegetables	8 (30)	13 (48)	3 (11)	3 (11)
Seed sprouts	9 (60)	6 (40)	0	0
Total	49	27	4	4

[a]Data compiled from various sources (21, 48, 156, 157, 159). These sources did not report outbreaks from single items of fresh produce caused by *S. aureus*, *L. monocytogenes*, or *Y. enterocolitica* in the United States during the given time period.
[b]Includes outbreaks from fruit juices.
[c]Percentage of outbreaks caused by a given pathogen within a given produce category.

UV: ultraviolet

associated with fresh leafy vegetables. These leafy vegetables consisted mostly of lettuce; the pathogenic *E. coli* was predominantly serotype O157:H7. The sources of the produce implicated in these different outbreaks vary widely in geographical location (48). This undermines the hypothesis that a given agricultural area harbors a certain type of pathogen and recurrently causes epidemics via the same type of produce. Rather, these trends suggest a relative specificity in the association of enteric pathogens with fruit and vegetables, and prompt important questions regarding the plant and bacterial factors that dictate this interaction, which can result in human disease.

ECOLOGY OF ENTERIC PATHOGENS IN THE PLANT ENVIRONMENT

The traditional view that human pathogens are defined by their ecological niche has been challenged by several studies. Certain bacterial species that commonly inhabit plant surfaces, including *Pseudomonas aeruginosa* (18, 25, 119), *Burkholderia cepecia* (115), *Erwinia* spp. (138), and *Enterococcus feacalis* (72, 105) have been shown to infect both plant and human tissue. The realization that enteric pathogens have a high incidence on fresh fruit and vegetables and that their presence on produce can lead to epidemics of foodborne illness has caused a further shift of paradigm regarding the niche specificity of human pathogens. The unexpected increase in produce-associated bacterial infections is indicative of a much more important role of plants as a secondary habitat for enteric pathogens than previously thought.

Fitness on Plant Surfaces

Plants as a nonhost environment. During their residence time on plants, enteric pathogens encounter harsh physicochemical conditions that fluctuate widely and rapidly over short periods of time (56). Such frequent and extreme changes, for example in temperature and osmotic conditions within the same day, may not be experienced by enteric pathogens in the animal and human gut. Also, unlike aerial plant surfaces, which are overall poor in nutrients (87), relatively aerobic, and exposed to UV (ultraviolet) radiation, the intestinal environment is replete in nutrients, anaerobic, and shielded from solar rays. The colon supports vigorous bacterial growth, with *E. coli* alone reaching between 10^6–10^9 cells per g colon content (131). While plants are considered as an overall hostile habitat for enteric pathogens, innovative studies in plant microbiology have shown that the spatial distribution of physicochemical conditions on leaf and root surfaces at the microscale is highly heterogeneous. For example, although sugars are present in small amounts on leaf surfaces and limit bacterial growth on leaves (170, 171), whole-cell bacterial biosensors for sucrose, fructose, and

glucose have revealed that these sugars are abundant in oases on leaves (81, 96); sucrose, fructose, and glucose are the dominant carbon sources in the phyllosphere of plants species examined so far (35, 94, 103). Distinct and localized spatial patterns of sucrose, amino acids, and nitrate abundance have also been mapped in the rhizosphere (34, 69). Similarly, microscopy of a bacterial sensor for water availability in the phyllosphere reported large variations in water potential between microsites on a given leaf surface, with only a subset of the cell population experiencing detectable water deprivation (4). In addition to starvation and osmotic and matric stress, bacteria on aerial plant surfaces have to cope with the effects of solar radiation. Besides the genes involved in pigmentation and in the repair of UV-damaged DNA, evidence also indicates that physical avoidance of UV radiation in shaded sites on leaves may be an important strategy for survival of bacteria on plant surfaces (66, 76). Therefore, it is hypothesized that because of the rugged topography of the plant landscape and its spatial heterogeneity in physicochemical conditions, enteric pathogens may encounter microsites on plant surfaces where conditions are favorable for their growth or survival.

Attachment. Attachment is the first step in the establishment of bacteria on the plant surface. The importance of this event in the contamination of produce is evidenced by several studies that suggest the involvement of specific determinants in the attachment of foodborne pathogens to plant tissue (6, 7, 46, 47, 146, 150, 154). As demonstrated with plant pathogens (127), several factors may be involved in the adhesion of enteropathogenic bacteria to plant tissue. These possibly include strategies that overlap with those used for attachment to animal and human epithelial cells, or that are common to those used by epiphytic or pathogenic bacteria for attachment to plants. For example, recent studies have shown that curli, long aggregative fimbriae that mediate binding to, and invasion of, epithelial cells in *E. coli* 0157:H7 (153) and *S. enterica* (141), are also implicated in the attachment of these pathogens to alfalfa sprouts (6, 71, 150). Conversely, the HecA adhesin in *Erwinia chrysanthemi*, which contributes to its virulence, aggregation on, and attachment to tobacco leaves, shares homology with hemagglutinins in animal pathogens (126). Thus, some bacterial determinants for attachment may have evolved from factors that bridge the plant and animal habitats. [For detailed information and further discussion, see (93, 136).]

Growth. Few studies have examined the ability of enteric pathogens to grow on the surface of live plants and the factors that affect their fitness in this ecological niche. O'Brien & Lindow (113) were the first to compare the population dynamics of leaf-associated bacteria with those of bacterial species that colonize the human intestine, in order to gain a better understanding of bacterial fitness traits required for colonization of the phyllosphere. Their growth chamber studies demonstrated that *S. enterica* Typhimurium and *E. coli* reached population sizes similar to those of *Pseudomonas syringae* in the bean and corn phyllosphere under constant wet conditions, whereas their population sizes were significantly lower than those of the bacterial epiphyte after incubation under dry conditions. Later studies on the colonization of the cilantro phyllosphere by a strain of *S. enterica* serovar Thompson isolated during an outbreak linked to cilantro revealed that the pathogen was less fit than the common epiphytic bacteria *Pseudomonas chlororaphis* and *Pantoea agglomerans* under wet conditions at 22°C (14). However, the competitiveness of *S. enterica* Thompson on wet cilantro leaves was significantly increased at plant incubation temperatures of 30°C and 37°C, enabling it to achieve rapidly higher population sizes than at 24°C. In growth chamber experiments using seeds planted in soil cores amended with contaminated manure, *S. enterica* was detected at harvest on the leaves of arugula and the roots of

GFP: green fluorescent protein

radish plants grown under conditions simulating a wet and warm "summer" environment, but not under those simulating a comparatively drier and cooler "spring" environment (109). The higher growth rates of the enteric pathogen at warm temperatures likely allow it to use a larger share of the nutrient resources that it can assimilate on the plant surface. Therefore, it is probable that free water from rain fall, dew, or irrigation, and concomitant warm temperatures enable *S. enterica* to undergo bursts of growth on plants in the field, thereby reaching the population sizes required to cause foodborne illness.

It appears, however, that optimal conditions of water availability and temperature are insufficient to allow enteric pathogens to maximally exploit plant surfaces as a habitat. Despite its higher growth rates and population sizes on warm and wet leaf surfaces, *S. enterica* Thompson achieved significantly lower final population sizes than the culturable resident bacteria in the cilantro phyllosphere (14). *S. enterica* and other enteric pathogens are likely not as well adapted as plant-colonizing bacteria to utilize the full spectrum of nutrients present on plant surfaces and, therefore, are limited by the amount and range of available nutrients that they can assimilate to thrive on plants. For example, unlike many plant-associated bacteria and *E. coli*, *S. enterica* is typically unable to assimilate sucrose (84), one of the main sugars present in leaf (87) and root exudates (31). It remains to be determined if this difference explains the relatively lower number of outbreaks caused by *S. enterica* on leaves compared to fruit (**Table 2**). On the other hand, *Campylobacter jejuni*, a thermophilic and microaerophilic foodborne pathogen, derives most of its energy from organic and amino acids and not from carbohydrates (38). *C. jejuni* survived poorly on plants, even at warm temperatures (13), which may be due to a lack of microsites with sufficient adequate nutrients and with low oxygen concentrations in that habitat. However, the pathogen had higher survival rates in the rhizosphere than in the phyllosphere, as well as on leaves that were wounded or contaminated with organic soil than on clean healthy leaves (13). These observations may partly explain the occasional presence of *C. jejuni* on fresh produce (78, 114) and the occurrence of produce-associated outbreaks of campylobacteriosis (67, 92), despite its generally weak epiphytic fitness.

As shown for most plant-adapted bacterial species introduced into the phyllosphere and then subjected to dry conditions, population sizes of *S. enterica* decline upon desiccation stress on leaves (14). This inability of the enteric pathogen to grow on dry leaves after a period of adaptation (14, 113) distinguishes it from a successful colonizer of leaf surfaces, *P. syringae*. This common plant pathogen has an extensive epiphytic phase and achieves detectable growth under dry conditions on host plants (8, 85). The enhanced ability of *P. syringae* to multiply on dry leaves may stem from its ingression into subsurface sites on the leaves of host plants (169), where it is shielded from the external water stress. It is unclear whether multiplication in/on dry plant surfaces is the primary determinant of bacterial fitness on plants. Many nonpathogenic plant-associated bacteria do not experience overall growth on dry leaves, presumably because they lack extensive endophytic lifestyles (129, 169). Similarly, *S. enterica* has not been observed frequently in natural openings on leaves, such as in the substomatal cavities (M.T. Brandl, unpublished data). Although growth in sites protected from desiccation confers a major advantage in bacterial colonization of the leaf habitat, plant colonists that are able to adapt to environmental stresses in exposed sites may have the opportunity to multiply when growth-conducive conditions resume.

Similar patterns of leaf colonization have been reported in epiphytic bacteria and in enteric pathogens. Inoculation of cilantro plants with cells of *S. enterica* labeled with the green fluorescent protein (GFP) revealed that this pathogen formed microcolonies (**Figure 2**), as well as aggregates, preferentially in the vein

area of the leaf (14). The localized growth of epiphytic bacteria at the base of glandular trichomes and in the vein region of cucumber and bean leaves has been described (79, 81, 99). The enhanced wettability of the surface of leaf veins (79) and the resulting increased availability of free water in that region (4), as well as the abundance of carbohydrates on veins and at the base of glandular trichomes (81), may explain the association of epiphytic bacteria (87), *S. enterica* (14), and *E. coli* O157:H7 (145) with these anatomical features of leaves.

Colonization of roots by rhizobacteria and foodborne pathogens can follow similar patterns also, as both have been observed to grow preferentially on root tips and/or at the root base where lateral roots emerge (10, 27, 36, 64, 120). This common growth pattern among bacterial species that have very distinct primary ecological niches may be correlated with the enhanced leakage of sugars and amino acids at these sites (69). Although enteric pathogens attach and aggregate at high densities in the root hair zone of seedlings in vitro (23, 47, 71), it remains to be determined if they also grow extensively in that region of the root in soil, especially since limited bacterial access to nitrogen nutrients in the root hair zone, due possibly to competitive uptake by the plant, has been reported (34).

Colonization of the entire plant, including the flowers and the seeds, was observed when *Arabidopsis thaliana* was grown in soil carefully irrigated with *S. enterica* or *E. coli* O157:H7 in a growth chamber (27). As is recognized already for plant pathogens, seed contamination may have important implications for the contamination cycle of enteric pathogens in crop production environments. In addition, seed contamination has greatly impacted the sprout production industry worldwide, as numerous geographically widespread outbreaks of foodborne illness have been linked to sprouts of alfalfa, radish, and mung bean seeds (**Table 2**) (48, 135).

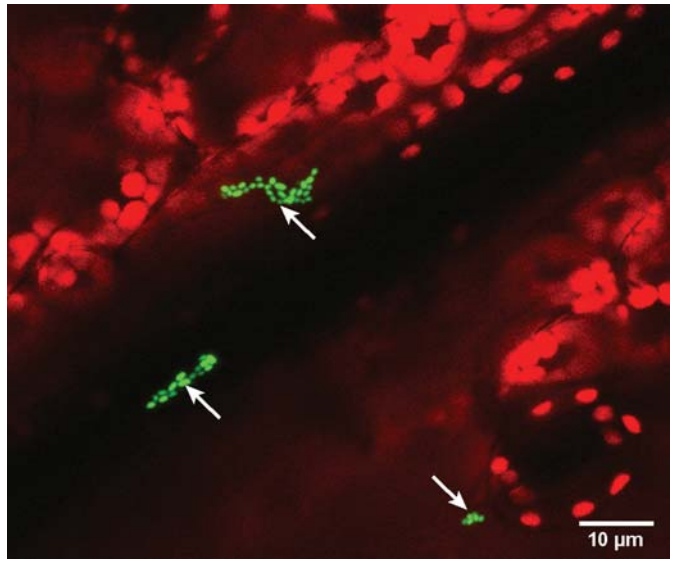

Figure 2

Confocal micrograph of colonies of GFP-labeled *S. enterica* (*white arrows*) observed on the vein (*large nonfluorescent area*) of a cilantro leaf two days after plant inoculation and incubation under warm and moist conditions. The red objects in the image are the autofluorescent chloroplasts of the epidermal cells surrounding the vein.

Survival. There has been a great interest in determining the persistence of foodborne pathogenic bacteria on fresh produce in the field in order to evaluate the public health risks associated with preharvest contamination of fresh produce. Several studies performed in the laboratory or in the field have demonstrated the ability of enteric pathogens to survive for prolonged periods of time on fruits and vegetables (39, 43, 61–63, 109, 137).

Ercolani (39), in some of the first evidence that enteric pathogens can persist on crops in the field, demonstrated that *S. enterica* Typhi was still detectable on mature lettuce plants that were inoculated at a young stage in the field. More recently, field studies conducted with an avirulent mutant of *S. enterica* Typhimurium and a nontoxigenic mutant of *E. coli* O157:H7 revealed that both pathogens were able to persist in the lettuce and parsley phyllosphere for at least three and six months, respectively, after the seedlings were planted and exposed to the pathogens via contaminated manure or irrigation water (61, 63). In

Salmonellosis: infection of the lining of the intestine with *Salmonella* spp., often caused by food poisoning

a similar field study, *S. enterica* Typhimurium also survived on field-grown radishes and carrots for 84 and 203 days, respectively, after the seeds were sown in soil contaminated with either compost or irrigation water (62). *E. coli* O157:H7 survived in the soil longer in field plots with long-season crops than in those with short-season crops (61). Additionally, the survival of *E. coli* O157:H7 in soil microcosms was strongly influenced by the presence of roots of particular plant species (43). These observations strongly suggest a role for the phyllosphere and the rhizosphere of crop plants in the overall persistence of enteric pathogens in agricultural environments.

In all of the field studies described above, the epiphytic population sizes of the enteric pathogens steadily declined after inoculation. However, sampling at broad time intervals, as done in these studies, does not allow for the detection of changes in bacterial populations sizes that may occur at shorter timescales due to the temporally variable conditions on plant surfaces. Hirano & Upper (55) reported large fluctuations in the population sizes of *P. syringae* in the bean phyllosphere under field conditions when sampling was performed at 2-h intervals. For two of the three sampling periods, diel variations in the population sizes of *P. syringae* did not correlate with short-term changes in weather conditions, indicating that general environmental conditions in the field do not accurately reflect the physicochemical conditions experienced by this bacterial species at the microscale on leaves. Although enteric pathogens do not have the same epiphytic fitness as *P. syringae*, it is likely that a subset of their population is able to multiply during the convergence of growth-optimal conditions in some microsites on plant surfaces. Such rare growth events may enable enteric pathogens either to achieve their infectious dose in a small proportion of a crop or to persist on a crop until harvest.

Low water activity is considered to be one of the main limitations of bacterial survival on plant surfaces. Enteric pathogens are exposed to osmotic stress in the animal and human gut (42), and have developed a range of mechanisms to adapt to such conditions (29). Additionally, *S. enterica* has a high tolerance to long-term desiccation stress in nonhost environments (33, 97). This may explain why *S. enterica* is the most common etiologic agent of foodborne illness linked to spices (163) and the recent occurrence of two salmonellosis outbreaks associated with dry raw almonds (60, 158). It was hypothesized that in at least one of these almond-linked outbreaks, *S. enterica* multiplied on the moist almond fruits on the orchard floor at harvest and survived on the dry kernels to which it had migrated (32, 152). It is telling of the pathogen's ability to cope with low water availability that the moisture content of dried nuts in storage is less than 6% (75). In growth chamber studies and under conditions of low relative humidity imposed for 24 h, *S. enterica* declined at rates similar to those of epiphytic bacteria on the dry leaf surface of cilantro plants, and was capable of resuming growth at significant rates upon reoccurrence of wet conditions on the leaves (11, 14). Thus, the tolerance of *S. enterica* to desiccation stress, combined with its potential to multiply under subsequent wet conditions may confer on this pathogen the ability to persist despite the high fluctuations in water availability on plant surfaces in the field. It implies also that even small populations of the pathogen that survive on crop plants before harvest may increase to infectious dose levels during growth-conducive conditions while the produce is stored or processed for consumption.

The factors involved in the survival of enteric pathogens to desiccation stress on plants remain to be elucidated. Epiphytic bacteria survive desiccation stress on leaves at higher rates as aggregated cells than as solitary cells, which suggests an important role for aggregate formation in bacterial tolerance to low water availability on plants (68, 98). Enteric pathogens also are capable of forming

aggregates under high moisture conditions in the phyllosphere (15) and the rhizosphere (23, 36, 47). In *S. enterica*, 54% of the cell population was located in aggregates composed of at least 128 cells, with some comprised of as many as 4096 cells, three days after inoculation onto moist cilantro leaves (15). These bacterial cells may be protected from the detrimental effect of rapid and frequent fluxes in water activity on plants while in the exopolymer matrix of aggregates.

A variety of genetic determinants that have a role in the stress tolerance and fitness of epiphytic bacteria on plants are present also in enteric pathogens and may serve similar purposes in these organisms in the phyllosphere and the rhizosphere. For example, both *S. enterica* and *E. coli* encode the sigma factor RpoS, which is involved in their adaptation to starvation and to a variety of stresses in vitro (52). Studies have suggested that RpoS increases rhizosphere colonization by *Pseudomonas fluorescens* in dry soil as well as its fitness on roots in moist soil in the field (140), and enhances the competitiveness of *Pseudomonas putida* on roots (95) and the fitness of *P. agglomerans* in the phyllosphere (M.T. Brandl & S.E. Lindow, unpublished data). Given that in *S. enterica*, starvation cross-protects against thermal, oxidative, and osmotic stresses via RpoS (42), and that bacteria experience frequent periods of starvation on plant surfaces, this sigma factor likely contributes to the fitness of *S. enterica* and possibly other enteric pathogens in the plant habitat. *S. enterica* and *E. coli* also harbor genes for cellulose production, a trait that is involved in their ability to form biofilms in vitro (128) and that has been associated with the fitness of *P. fluorescens* in the rhizosphere and phyllosphere of sugar beet (44). Furthermore, genes involved in tolerance to UV radiation, an important phenotype for microorganisms that inhabit aerial plant surfaces, have been identified in both epiphytic bacteria and enteric pathogens. The *rulAB* genes for mutagenic DNA repair imparted enhanced survival to UV-B radiation on *P. syringae* on plants in the growth chamber (143), and to solar radiation on bean leaves in the field (142). Several homologues of *rulAB* exist in *E. coli* and *S. enterica*, in which they share a molecular function similar to that of *rulAB* in *P. syringae* (111, 143, 172). Whether these homologues are part of the strategy employed by *E. coli* and *S. enterica* to survive exposure to solar radiation on plants remains to be determined. Several other traits have been linked to bacterial colonization of plants, including lipopolysaccharide and exopolysaccharide production, motility, and chemotaxis (87, 91). The phenotypes described above provide a basis on which to evaluate the role of similar characteristics in the fitness of enteric pathogens on plant surfaces.

Interactions of Enteric Pathogens with the Plant Microflora

As human pathogens land in the plant environment, they have to compete for resources in this ecological niche with members of the resident microflora that are highly adapted to that habitat. For example, reduction of the microflora of broad-leaved endive by chemical disinfection subsequently allowed for increased colonization by *L. monocytogenes* (20). Extensive disinfection of fresh produce could therefore leave a vacuum with little competition against enteric pathogens contaminating the produce after it has been sanitized. On the other hand, Monier & Lindow (100) have demonstrated that the fate of immigrant cells of nonpathogenic plant-associated bacteria in the phyllosphere is highly dependent on whether they land as single cells or in an indigenous aggregate before exposure to desiccation stress. Similarly, interactions of enteric pathogens with the resident plant microflora may be beneficial to their growth or survival after they immigrate onto plants. This aspect of their ecology on plants could prove to be a major factor in their persistence on crops and in their potential to cause disease via contaminated fruit and vegetables, as well as in their adaptation to the plant habitat over evolutionary times.

Growth promotion and inhibition. Little information exists regarding the interaction of human pathogens with the microflora of healthy plants. However, several studies have investigated the role of plant disease in the contamination of produce with foodborne pathogens. In a survey of produce at the marketplace, Wells & Butterfield (168) observed twice the incidence of *Salmonella* species on fruit and vegetables affected by soft rot as on healthy produce. Furthermore, they showed that the population sizes of *Salmonella* on potato, carrot, and bell pepper disks coinoculated with the soft rot pathogen *Erwinia carotovora* or *Pseudomonas viridiflava* were ten- and threefold higher, respectively, than when inoculated with the enteric pathogen alone. Soft rot also had a positive effect on the population sizes of *L. monocytogenes* on endive leaves (19).

It is presumed that the damaged cells in rotten plant tissue leak abundant nutrients that would not otherwise be available to enteric pathogens on healthy plant surfaces. This assumption is supported by the observation that the growth of enteric pathogens is also enhanced in plant tissue that is simply injured mechanically (17, 175). In addition to nutrient leakage out of the damaged plant cells during disease, the degradation of plant tissue by plant pathogens may offer enteric pathogens a broadened spectrum of nutrients from which to derive their energy. However, not all plant pathogens affect the colonization of plant tissue by enteric pathogens equally. For example, when *S. enterica* was coinoculated with *E. chrysanthemi* or *P. viridiflava*, its population sizes on the diseased leaves of cilantro plants were more highly correlated with those of *E. chrysanthemi* than with those of *P. viridiflava* (12). Moreover, both the growth rate and final population sizes of *S. enterica* were greater in *E. chrysanthemi*-infected leaves than in leaves infected with *P. viridiflava*. In a separate study, the growth of *L. monocytogenes* was strongly inhibited in the presence of *Pseudomonas fluorescens* and *P. viridiflava*, whereas its growth was not affected, either negatively or positively, by the presence of *Erwinia carotovora* or *Xanthomonas campestris*, after coinoculation with one of these four pathogens onto potato tuber slices (82). Competition for iron via production of siderophores was suggested as a possible mechanism for the antagonism by the pseudomonads in this study (82). Conversely, in other situations, the growth of enteric pathogens may be enhanced by their heterologous utilization of siderophores produced by plant-associated microorganisms or by the plants themselves. Utilization of phytosiderophores by *Pseudomonas* in the rhizosphere has been reported (74). Additionally, Loper & Henkel (88) demonstrated that the iron availability to *Pseudomonas putida* in the cucumber rhizosphere was increased by its utilization of siderophores produced by *Enterobacter cloacae*. Members of the Enterobacteriaceae, including *S. enterica* and *E. coli*, produce a variety of siderophores (117). It remains to be determined whether plant-associated bacteria in this family share iron acquisition systems with enteric pathogens in the plant habitat.

Role of aggregates. The investigation of biofilms in the phyllosphere by Morris and coworkers (104) has spurred interest in the role of biofilms in the ecology of microbes on plant surfaces. Such large mixed microbial aggregates have since been observed on a variety of naturally colonized plant surfaces (40, 41, 68, 123). Considering that a significant proportion of the cell population of epiphytic bacteria (99) and *S. enterica* (15) on plants is located in large aggregates, these microbial assemblages may play an important role, not only in the protection of bacteria cells within the polymer matrix of the aggregates, but also in their interaction with various members of that community. Complex patterns of microbial interactions that are dictated by species composition have been observed within mixed aggregates of plant-associated bacteria on leaves (101). Likewise, the species and strain of pre-existing epiphytic bacteria had a strong influence on the survival

of *S. enterica* and *E. coli* O57:H7 inoculated into the phyllosphere (118), indicating that the outcome of the interaction between enteric pathogens and plant microflora is heavily dependent on the identity of the microorganisms forming the aggregate.

Although antagonism may occur within heterogeneous aggregates, other interactions may be beneficial to the fitness of enteric pathogens on plants. For example, cross-talk via quorum-sensing signals produced by epiphytic or plant pathogenic bacteria may benefit enteric pathogens present in mixed aggregates on healthy or diseased plants. Acyl-homoserine lactones (AHLs), which are produced by several species of epiphytic and plant pathogenic bacteria (164), have been detected in spoiled produce (122). Although *S. enterica* and *E. coli* do not synthesize AHLs, *S. enterica* possesses a *luxR* homologue (*sdiA*) by which it responds to AHLs from other bacterial species in its vicinity (2). One could hypothesize that communication between *S. enterica* and plant bacteria via AHLs upregulates determinants that influence the behavior of the human pathogen on plants.

Although *S. enterica* produces the autoinducer-2 (AI-2) molecule (144), the proposed universal signal for quorum sensing (132), no evidence was found that production of AI-2 in *S. enterica*, or in epiphytic bacteria coinoculated with the AI-2-deficient mutant of *S. enterica*, had a role in the population size that the human pathogen reached in the phyllosphere (15). Additionally, the frequency distribution of the size of aggregates formed by *S. enterica* on leaves did not differ significantly from that of its AI-2-deficient mutant, suggesting that AI-2 does not contribute to its aggregate formation on plants. On the contrary, AI-2 production contributed greatly to the ability of *S. enterica* to colonize the chicken intestine, indicating a niche-specific role for the AI-2 system in this pathogen (15).

Conjugal transfer. Bacterial aggregation on plant surfaces provides the close contact required for conjugal transfer of genetic material between bacteria. The transfer ratio of the TOL plasmid between *P. putida* strains was 30-fold higher on leaves than on membrane filters, and transconjugants were observed primarily in the aggregates at the junction of epidermal cells and in the substomatal cavities of the leaves (112). These observations may help explain the high rates of acquisition of a mercury resistance-encoding plasmid by *P. fluorescens* from the indigenous microflora in the sugar beet phyllosphere (83). The transfer of the mercury resistance plasmid coincided with particular conditions on the plant surface, suggesting that selective pressure favored transconjugants because of particular fitness traits conferred by the plasmid (5). Genetic transfer of R factors between various plant-associated bacterial species and *S. enterica* Typhimurium has been demonstrated in vitro (24). As enteric pathogens interact with other bacteria species in the plant environment, horizontal gene transfer provides a mechanism for the enteric pathogens to gain traits that confer higher fitness in that habitat. Of particular concern is the acquisition of antibiotic resistance genes by enteric pathogens during their residence on agricultural crops. Antibiotic treatments for the protection of crops against plant pathogenic bacteria could place sufficient selective pressure for the maintenance of such determinants in populations of human pathogens outside of their hosts.

Interactions with plant microflora other than bacteria. Fungi are important members of microbial consortia on plants surfaces. Of particular interest in the ecology of enteric pathogens on fruit and vegetables is their interaction with fungi that cause postharvest decay. Similar to observations made with plant pathogenic bacteria, the outcome of the interaction between plant pathogenic fungi and enteric pathogens depends on the fungal species. Whereas *L. monocytogenes* and *E. coli* O157:H7 proliferated during infection of apple fruit tissue with *Glomerella cingulata*, the enteric pathogens declined in wounds colonized by

AHL: acyl-homoserine lactone

Penicillium expansum; this differential effect presumably resulted from specific physiological changes in the rotten tissue, with a shift in pH from ca. 4.0 to 7.0 and 4.7 to 3.7 in the presence of *G. cingulata* and *P. expansum*, respectively (26, 125). The enhanced growth of *S. enterica* in raw tomatoes infected with *Cladosporium* spp. or *Alternaria alternata* (165), or with proteolytic yeasts (166) has been reported; like in the apple studies, it was suggested that growth promotion of the enteric pathogen was mediated by an increase to a more favorable pH of the pulp tissue. In addition to altering the physicochemical conditions in the plant tissue, fungi may serve as vectors that spread the enteric pathogens onto or into plants. The attachment of various bacterial species to fungi has been demonstrated previously in studies of biocontrol of plant pathogenic fungi with bacteria (80, 110, 176), and of mycorrhizal associations with bacteria (3 and references therein). Microscopic examination of *S. enterica* cells inoculated onto cilantro plants revealed the association of this pathogen with the hyphae of indigenous fungi present on the leaf surface (**Figure 3**) (M.T. Brandl, unpublished data). The enhanced recovery of *S. enterica* cells in the tissue below cantaloupe wounds coinoculated with the pathogen and *C. cladosporioides* or *P. expansum* suggests that fungi may facilitate the migration of enteric pathogens onto and into plant tissue (124).

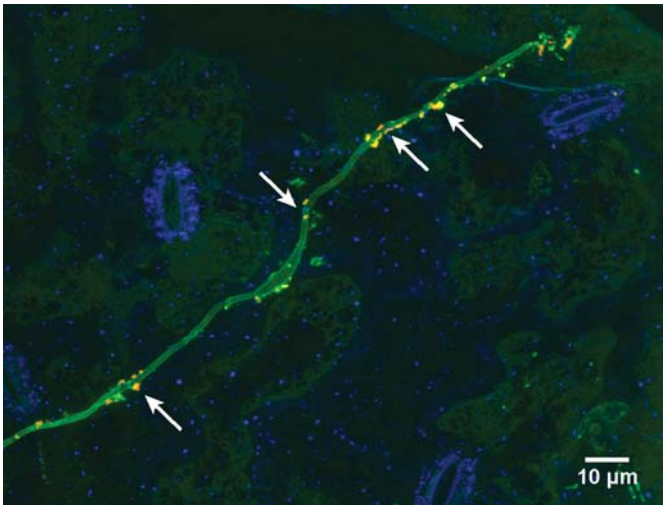

Figure 3

Confocal micrograph of dsRed-labeled *S. enterica* cells (*white arrows*) adhering to the hypha of an indigenous fungus that colonized the cilantro leaf surface. The cells were observed two days after inoculation of the plants with the enteric pathogen and incubation under warm and moist conditions. Both *S. enterica* and the fungus were stained with the fluorescein-labeled Concanavalin A lectin. The combination of the fluorescein and dsRed signals imparts yellow-orange fluorescence onto *S. enterica* cells. Note the absence of *S. enterica* cells in the vicinity of the fungus, which suggests that the enteric pathogen was vectored by the fungus from a distant location on the leaf. The autofluorescence of the leaf chloroplasts was assigned the pseudocolor blue. Bar, 10 µm.

Protozoa are present in high number on fruit and vegetables, including on postharvest produce (S.G. Berk & M.T. Brandl, unpublished data). The interaction of protozoa with bacteria has been examined mostly in the rhizosphere and from the traditional perspective of predator-prey relationships. Insightful investigations into the role of *Acanthamoeba* spp. in the disease cycle of *Legionella pneumophila* (1) and increasing concerns over the survival and dissemination of food-borne pathogens in the environment have sparked a new interest in the interaction of human pathogens with protozoa. Brandl et al. (16) demonstrated that *S. enterica* and *E. coli* O157:H7 resist digestion by *Tetrahymena* at particular ratios of the bacteria to the protist, and are released from *Tetrahymena* as viable cells encased in vesicles. The encased cells had higher survival in water and in low hypochlorite solutions than cells remaining free in suspension, suggesting that the membrane surrounding them in the vesicles offered protection from environmental stress. It is hypothesized that such interactions may take place also on plant surfaces, particularly when conditions of high moisture on plants coincide with bacterial bloom.

INFECTIOUS DOSE IN RELATION TO PLANTS AS A FOOD MATRIX

An important paradox about produce-associated outbreaks of food-borne illness is the presumed high infectious dose of some

pathogens and the relatively small number of cells of any enteric pathogens that is hypothesized to be present on plant crops. Levels of contaminants are low presumably also on plant-derived foods that have low water activity (e.g., nuts and spices). Nevertheless, such foods have been implicated in foodborne illness (60, 163). This prompts the question of whether the plant environment affects the physiology of enteric pathogens such that fewer bacterial cells are required to cause disease in humans.

The low pH of the stomach is thought to be the first barrier against infection with foodborne pathogens. The adaptation of enteric pathogens to acidic conditions on cut or diseased plant tissue may help them to overcome the low pH of the human gastric environment and decrease their infectious dose. *S. enterica* cells that were inoculated onto fresh-cut produce surfaces had increased survival to acid challenge (pH 3.0, 37°C, 2 h) (9). In addition to the acidity of some fresh-cut fruit and vegetables, the presence of soft rot on produce may induce the acid tolerance of enteric pathogens via the acidic intercellular apoplastic fluids resulting from the degradation of the plant cell wall by soft rot pathogens (106). Likewise, the exposure of enteric pathogens to plant antimicrobial peptides released during plant cell damage or infection by plant pathogens may upregulate determinants that enhance their ability to withstand the first line of defense in the human host. A homologue of the *sap* operon, which confers resistance to host antimicrobial peptides and has a role in virulence in *S. enterica* (116), is implicated also in the resistance of *E. chrysanthemi* to plant antimicrobial compounds and in its virulence on plants (89, 90). This suggests the possibility that the *sap* operon is induced in *S. enterica* by plant antimicrobial peptides in damaged/rotten plant tissue, and, consequently, enables the pathogen to better resist early human host defenses.

The expression of virulence/pathogenicity determinants in enteric pathogens during their residence on plants could be paramount to their infectious dose from contaminated produce because it may prime the pathogens for attack against the human host epithelial cells. Barak et al. (6) demonstrated that curli have a role in binding of *S. enterica* to alfalfa sprouts, indicating that virulence factors are produced by and are of benefit to enteric pathogens on plant surfaces. The commonality in some pathogenicity or virulence factors involved in bacterial infection of plants and humans has been demonstrated (18, 139). These include components of the type III secretion system (TTSS), which has been identified in both human and plant pathogens, as well as in nonpathogenic plant-associated bacteria (49, 65). The TTSS mediates epiphytic colonization of the phyllosphere in *P. syringae* pv. *syringae* (54) and also of the rhizosphere in *P. fluorescens* (65). Surprisingly, mutations in components of the TTSS encoded by the pathogenicity island 1 of *S. enterica* (SPI1) reduced the ability of the pathogen to endophytically colonize alfalfa roots in vitro, suggesting that the SPI1-encoded TTSS is expressed and assembled in *S. enterica* in plant roots (59). Furthermore, colonization of *A. thaliana* by this enteric pathogen was affected by plant defense responses that involve recognition of *S. enterica* flagella and SPI1-encoded TTSS effectors (59). Despite its apparent role in colonization, the significance of TTSS synthesis in enteric pathogens on crop plants to dose-response relationships in the human host remains to be investigated.

TTSS: type III secretion system

SPI-1: *Salmonella* pathogenicity island 1

PERSPECTIVES AND FUTURE DIRECTIONS

Evidence is emerging that enteropathogenic bacteria, although not as fit in the plant habitat as common plant-associated bacterial species, have the ability to grow and persist on crop plants. This versatile life style may explain the remarkable incidence of foodborne illness associated with the contamination of agricultural crops. Still unclear, however, is whether the relative fitness of enteric pathogens on

plants in the field is sufficient to explain the etiology of outbreaks linked to fresh produce, or if bacterial proliferation in the postharvest environment is necessary for the enteric pathogens to achieve infectious dose levels on produce. It could be argued that the bacterial population size of the enteric pathogen on produce does not constitute the sole determinant of infectivity in the human host. Rather, the physiology and the interactions of enteric pathogens on plants may enhance not only their survival in that habitat but also their ability to infect humans.

Despite recent advances in our understanding of the ecology of foodborne pathogens on plant surfaces, the fundamental bacterial and plant factors that mediate the ability of enteric pathogens to colonize plants, to resist traditional methods of decontamination, and to cause human illness via fresh produce as a food matrix are poorly understood. As early research efforts focused on acquiring information to shed the prevailing dogma that enteric pathogens lacked the epiphytic fitness to be etiologic agents of produce-linked outbreaks, future studies should delve into the fundamental biology of enteric pathogens on plants. This should be greatly facilitated by the large amount of genomic and proteomic data that are available on these organisms may well shed light on the role of their numerous genes that still have unknown function.

Future research should also draw from the wealth of knowledge acquired from studies in plant pathology and microbial ecology, to formulate hypotheses on plant-microbe and microbe-microbe interactions relevant to the pre- and postharvest colonization of plants by enteric pathogens. Coincidentally, enhanced knowledge of the comparative behavior of enteric pathogens and plant pathogenic or epiphytic bacteria on plants will enlighten us on the plant and bacterial factors that drive their evolution in that habitat. From a pragmatic perspective, fundamental studies will allow for a better understanding of the epidemiology of foodborne illness linked to produce and for the identification of critical control points to develop good agricultural practices and interventions based on the integration of multiple prevention and control strategies into hurdle technologies.

SUMMARY POINTS

1. The number of outbreaks of foodborne disease linked to the consumption of fresh produce has increased since the early 1990s.
2. *S. enterica* and *E. coli* O157:H7, both previously associated with illness from foods of animal origin, cause the highest proportion of fresh produce-linked epidemics that have a known etiologic agent.
3. Surveillance data have provided strong evidence for the presence of enteropathogenic bacteria on fresh fruit and vegetables.
4. Not all enteric pathogen species are ecological generalists. Large differences exist among various enteric pathogens in their ability to attach to and colonize plant surfaces, and particular phenotypes play a role in some of these differences.
5. Although the fitness of *S. enterica* on plants is relatively weaker than that of common plant-associated bacteria, this human pathogen can grow on plant surfaces under conditions of high moisture and warm temperature, two factors that affect its competitiveness in that habitat.
6. Several studies performed in growth chambers and in the field have demonstrated that *S. enterica* and *E. coli* inoculated at planting time persist on crops for prolonged periods of time, including until harvest.

7. Diseased plant tissue may provide a nutrient-rich and protected ecological niche for enteric pathogens. However, this opportunity for growth is dictated by the nature of their interactions with the resident plant microflora.
8. The expression in enteric pathogens, of survival determinants or of virulence traits in the plant habitat, may modulate dose-response relationships in the human host and allow for foodborne illness to occur with low infectious doses.

ACKNOWLEDGMENTS

I thank Ronald Amundson, Magalie Guilhabert, Robert Mandrell, and Beatriz Quiñones for helpful comments on the manuscript. Work in my laboratory is funded by U.S. Department of Agriculture, Agriculture Research Service CRIS projects 201-5325-210-40 and 201-5325-210-41.

LITERATURE CITED

1. Abu Kwaik Y, Gao LY, Stone BJ, Venkataraman C, Harb OS. 1998. Invasion of protozoa by *Legionella pneumophila* and its role in bacterial ecology and pathogenesis. *Appl. Environ. Microbiol.* 64:3127–33
2. Ahmer BMM. 2004. Cell-to-cell signaling in *Escherichia coli* and *Salmonella enterica*. *Mol. Microbiol.* 52:933–45
3. Artursson V, Jansson JK. 2003. Use of bromodeoxyuridine immunocapture to identify active bacteria associated with arbuscular mycorrhizal hyphae. *Appl. Environ. Microbiol.* 69:6208–15
4. Axtell CA, Beattie GA. 2002. Construction and characterization of a *proU-gfp* transcriptional fusion that measures water availability in a microbial habitat. *Appl. Environ. Microbiol.* 68:4604–12
5. Bailey MJ, Rainey PB, Zhang XX, Lilley AK. 2002. Population dynamics, gene transfer and gene expression in plasmids, the role of the horizontal gene pool in local adaptation at the plant surface. See Ref. 87a, pp. 173–91
6. **Barak JD, Gorski L, Naraghi-Arani P, Charkowski AO. 2005. *Salmonella enterica* virulence genes are required for bacterial attachment to plant tissue. *Appl. Environ. Microbiol.* 71:5685–91**
7. Barak JD, Whitehand LC, Charkowski AO. 2002. Differences in attachment of *Salmonella enterica* serovars and *Escherichia coli* O157:H7 to alfalfa sprouts. *Appl. Environ. Microbiol.* 68:4758–63
8. Beattie GA, Lindow SE. 1994. Comparison of the behavior of epiphytic fitness mutants of *Pseudomonas syringae* under controlled and field conditions. *Appl. Environ. Microbiol.* 60:3799–808

8a. Beier RC, Pillai SD, Philips TD, Ziprin RL, eds. 2004. *Preharvest and Postharvest Food Safety: Contemporary Issues and Future Directions*. Ames, IA: Blackwell

9. Bhagwat AA. 2006. Microbiological safety of fresh-cut produce: Where are we now? See Ref. 93a, pp. 121–65
10. Bloemberg GV, Wijfjes AH, Lamers GE, Stuurman N, Lugtenberg BJ. 2000. Simultaneous imaging of *Pseudomonas fluorescens* WCS365 populations expressing three different

First paper to describe the requirement of a known pathogen attachment and virulence factor in the human host for the attachment of the enteric pathogen to a plant surface.

autofluorescent proteins in the rhizosphere: new perspectives for studying microbial communities. *Mol. Plant-Microbe Interact*. 13:1170–76
11. Brandl MT. Human pathogens and the health threat of the phyllosphere. In *Microbial Ecology of Aerial Plant Surfaces*, ed. M Bailey, P Spencer-Philips. Cambridge, UK: CABI Publ. In Press
12. Brandl MT. 2004. Fitness of *Salmonella enterica* in the phyllosphere. *Phytopathology* 94(Suppl.):128
13. Brandl MT, Haxo AF, Bates AH, Mandrell RE. 2004. Comparison of survival of *Campylobacter jejuni* in the phyllosphere with that in the rhizosphere of spinach and radish plants. *Appl. Environ. Microbiol*. 70:1182–89

This study investigated the factors that affect the fitness of *Salmonella enterica* in the phyllosphere.

14. Brandl MT, Mandrell RE. 2002. Fitness of *Salmonella enterica* serovar Thompson in the cilantro phyllosphere. *Appl. Environ. Microbiol.* 68:3614–21
15. Brandl MT, Miller WG, Bates AH, Mandrell RE. 2005. Production of autoinducer 2 in *Salmonella enterica* serovar Thompson contributes to its fitness in chickens but not on cilantro leaf surfaces. *Appl. Environ. Microbiol*. 71:2653–62
16. Brandl MT, Rosenthal BM, Haxo AF, Berk SG. 2005. Enhanced survival of *Salmonella enterica* in vesicles released by a soilborne *Tetrahymena* species. *Appl. Environ. Microbiol*. 71:1562–69
17. Campbell VJ, Mohle-Boetani J, Reporter R, Abbott S, Farrar J, et al. 2001. An outbreak of *Salmonella* serotype Thompson associated with fresh cilantro. *J. Infect. Dis.* 183:984–87
18. Cao H, Baldini R, Rhame LG. 2001. Common mechanisms for pathogens of plants and animals. *Annu. Rev. Phytopathol*. 39:259–84
19. Carlin F, Nguyen-The C, Abreu da Silva A. 1995. Factors affecting the growth of *Listeria monocytogenes* on minimally processed fresh endive. *J. Appl. Bacteriol*. 78:636–46
20. Carlin F, Nguyen-The C, Morris CE. 1996. Influence of background flora on *Listeria monocytogenes* on minimally processed fresh broad-leaved endive (*Cichorium endivia* var. latifolia). *J. Food Prot*. 59:698–703
21. Cent. Sci. Public Interest. 2002. Outbreak Alert! Closing the gaps in our federal food-safety net. Washington, DC. **http://www.cspinet.org/Report**
22. Cent. Sci. Public Interest. 2005. Outbreak Alert! Closing the gaps in our federal food-safety net. Washington, DC. **http://www.cspinet.org/Report**
23. Charkowski AO, Barak JD, Sarreal CZ, Mandrell RE. 2002. Differences in growth of *Salmonella enterica* and *Escherichia coli* O157:H7 on alfalfa sprouts. *Appl. Environ. Microbiol*. 68:3114–20
24. Cho JJ, Panopoulos NJ, Schroth MN. 1975. Genetic transfer of *Pseudomonas aeruginosa* R factors to plant pathogenic *Erwinia* species. *J. Bacteriol*. 122:192–98
25. Cho JJ, Schroth MN, Kominos SD, Green SK. 1975. Ornamental plants as carriers of *Pseudomonas aeruginosa*. *Phytopathology* 65:425–31
26. Conway WS, Leverentz B, Saftner RA, Janisiewicz WJ, Sams CE, Leblanc E. 2000. Survival and growth of *Listeria monocytogenes* on fresh-cut apple slices, and its interaction with *Glomerella cingulata* and *Penicillium expansum*. *Plant Dis*. 84:177–81
27. Cooley M, Miller W, Mandrell R. 2003. Colonization of *Arabidopsis thaliana* with *Salmonella enterica* or enterohemorrhagic *Escherichia coli* O157:H7 and competition by an *Enterobacter asburiae*. *Appl. Environ. Microbiol*. 69:4915–26
28. Creel R. 1912. Vegetables as a possible factor in the dissemination of typhoid fever. *Public Health Rep*. 27:187–93
29. Csonka LN, Epstein W. 1996. Osmoregulation. See Ref. 109, pp. 1210–23

30. Cummings K, Barrett E, Mohle-Boetani JC, Brooks JT, Farrar J, et al. 2001. A multistate outbreak of *Salmonella enterica* serotype Baildon associated with domestic raw tomatoes. *Emerg. Infect. Dis.* 7:1046–48
31. Curl EA, Truelove B. 1986. Root exudates. In *The Rhizosphere*, ed. B Yaron, pp. 55–92. Berlin: Springer-Verlag
32. Danyluk MD, Brandl MT, Harris LJ. 2005. *Migration of* Salmonella enteritidis *PT 30 through almond hulls and shells*. Presented at Annu. Meet. Int. Assoc. Food Prot., Baltimore, MD (Abstr. T1–06)
33. Davies RH, Wray C. 1995. Observations of disinfection regimens used on *Salmonella enteritidis* infected poultry units. *Poultry Sci*. 74:638–47
34. DeAngelis KM, Ji P, Firestone MK, Lindow SE. 2005. Two novel bacterial biosensors for detection of nitrate availability in the rhizosphere. *Appl. Environ. Microbiol*. 71:8537–47
35. Derridj S. 1996. Nutrients on the leaf surface. In *Aerial Plant Surface Microbiology*, ed. CE Morris, PC Nicot, C Nguyen-The, pp. 25–42. New York: Plenum
36. Dong Y, Iniguez AL, Ahmer BM, Triplett EW. 2003. Kinetics and strain specificity of rhizosphere and endophytic colonization by enteric bacteria on seedlings of *Medicago sativa* and *Medicago truncatula*. *Appl. Environ. Microbiol*. 69:1783–90
37. Dowd SE, Thurston-Enriquez JA, Brashears M. 2004. Environmental reservoirs and transmission of foodborne pathogens. See Ref. 8a, pp. 161–71
38. Elharrif Z, Megraud F. 1986. Characterization of thermophilic *Campylobacter*: I. Carbon substrate utilization tests. *Curr. Microbiol*. 13:117–22
39. Ercolani GL. 1979. Differential survival of *Salmonella typhi*, *Escherichia coli*, and *Enterobacter aerogenes* on lettuce in the field. *Zentralbl. Bakteriol. Naturwiss*. 134:402–11
40. Fett WF. 2000. Naturally occurring biofilms on alfalfa and other types of sprouts. *J. Food Prot*. 63:625–32
41. Fett WF, Cooke PH. 2003. Scanning electron microscopy of native biofilms on mung bean sprouts. *Can. J. Microbiol*. 49:45–50
42. Foster JW, Spector MP. 1995. How *Salmonella* survive against the odds. *Annu. Rev. Microbiol*. 49:145–74
43. Gagliardi JV, Karns JS. 2002. Persistence of *Escherichia coli* O157:H7 in soil and on plant roots. *Environ. Microbiol*. 4:89–96
44. Gal M, Preston GM, Massey RC, Spiers AJ, Rainey PB. 2003. Genes encoding a cellulosic polymer contribute toward the ecological success of *Pseudomonas fluorescens* SBW25 on plant surfaces. *Mol. Ecol*. 12:3109–21
45. Gerba CP, McLeod JS. 1976. Effect of sediments on the survival of *Escherichia coli* in marine waters. *Appl. Environ. Microbiol*. 32:114–20
46. Gorski L, Palumbo JD, Mandrell RE. 2003. Attachment of *Listeria monocytogenes* to radish tissue is dependent upon temperature and flagellar motility. *Appl. Environ. Microbiol*. 69:258–66
47. Gorski L, Palumbo JD, Nguyen KD. 2004. Strain-specific differences in the attachment of *Listeria monocytogenes* to alfalfa sprouts. *J. Food Prot*. 67:2488–95
48. Harris LJ, Farber JN, Beuchat LR, Parish ME, Suslow TV, et al. 2003. Outbreaks associated with fresh produce: incidence, growth, and survival of pathogens in fresh and fresh-cut produce. *Comp. Rev. Food Sci. Food Saf*. 2(Suppl.):78–141
49. He SY. 1998. Type III protein secretion systems in plant and animal pathogenic bacteria. *Annu. Rev. Phytopathol*. 36:363–92
50. Hedberg CW, Angulo FJ, White KE, Langkop CW, Schell WL, et al. 1999. Outbreaks of salmonellosis associated with eating uncooked tomatoes: implications for public health. *Epidemiol. Infect.* 122:385–93

51. Hendricks CW. 1971. Increased recovery rate of salmonellae from stream bottom sediments versus surface waters. *Appl. Microbiol.* 21:379–80
52. Hengge-Aronis R. 1996. Regulation of gene expression during entry into stationary phase. See Ref. 109a, pp. 1497–512
53. Himathongkham S, Bahari S, Riemann H, Cliver D. 1999. Survival of *Escherichia coli* O157:H7 and *Salmonella* Typhimurium in cow manure and cow manure slurry. *FEMS Microbiol. Lett.* 178:251–57
54. Hirano SS, Charkowski AO, Collmer A, Willis DK, Upper CD. 1999. Role of the Hrp type III protein secretion system in growth of *Pseudomonas syringae* pv. *syringae* B728a on host plants in the field. *Proc. Natl. Acad. Sci. USA* 96:9851–56
55. Hirano SS, Upper CD. 1989. Diel variation in population size and ice nucleation activity of *Pseudomonas syringae* on snap bean leaflets. *Appl. Environ. Microbiol.* 55:623–30
56. Hirano SS, Upper CD. 2000. Bacteria in the leaf ecosystem with emphasis on *Pseudomonas syringae*—a pathogen, ice nucleus, and epiphyte. *Microbiol. Mol. Biol. Rev.* 64:624–53
57. Horby PW, O'Brien SJ, Adak GK, Graham C, Hawker JI, et al. 2003. A national outbreak of multi-resistant *Salmonella enterica* serovar Typhimurium definitive phage type (DT) 104 associated with consumption of lettuce. *Epidemiol. Infect.* 130:169–78
58. Hutchison ML, Walters LD, Moore A, Crookes KM, Avery SM. 2004. Effect of length of time before incorporation on survival of pathogenic bacteria present in livestock wastes applied to agricultural soil. *Appl. Environ. Microbiol.* 70:5111–18
59. Iniguez AL, Dong Y, Carter HD, Ahmer BM, Stone JM, Triplett EW. 2005. Regulation of enteric endophytic bacterial colonization by plant defenses. *Mol. Plant-Microbe Interact.* 18:169–78
60. Isaacs S, Aramini J, Ciebin B, Farrar JA, Ahmed R, et al. 2005. An international outbreak of salmonellosis associated with raw almonds contaminated with a rare phage type of *Salmonella enteritidis*. *J. Food Prot.* 68:191–98
61. Islam M, Doyle MP, Phatak SC, Millner P, Jiang X. 2004. Persistence of enterohemorrhagic *Escherichia coli* O157:H7 in soil and on leaf lettuce and parsley grown in fields treated with contaminated manure composts or irrigation water. *J. Food Prot.* 67:1365–70
62. Islam M, Morgan J, Doyle MP, Phatak SC, Millner P, Jiang X. 2004. Fate of *Salmonella enterica* serovar Typhimurium on carrots and radishes grown in fields treated with contaminated manure composts or irrigation water. *Appl. Environ. Microbiol.* 70:2497–502
63. Islam M, Morgan J, Doyle MP, Phatak SC, Millner P, Jiang X. 2004. Persistence of *Salmonella enterica* serovar Typhimurium on lettuce and parsley and in soils on which they were grown in fields treated with contaminated manure composts or irrigation water. *Foodborne Path. Dis.* 1:27–35
64. Jablasone J, Warriner K, Griffiths M. 2005. Interactions of *Escherichia coli* O157:H7, *Salmonella* Typhimurium and *Listeria monocytogenes* plants cultivated in a gnotobiotic system. *Int. J. Food Microbiol.* 99:7–18
65. Jackson RW, Preston GM, Rainey PB. 2005. Genetic characterization of *Pseudomonas fluorescens* SBW25 *rsp* gene expression in the phytosphere and in vitro. *J. Bacteriol.* 187:8477–88
66. Jacobs JL, Carroll TL, Sundin GW. 2005. The role of pigmentation, UV radiation tolerance, and leaf colonization strategies in the epiphytic survival of phyllosphere bacteria. *Microb. Ecol.* 49:104–13

First demonstration that *Salmonella* engages in plant-microbe interactions in plants, and that this affects its plant colonization ability.

References 61–63 describe the persistence of enteric pathogens in soil and on various crops in the field from planting until past harvest time.

67. Jacobs-Reitsma W. 2000. *Campylobacter* in the food supply. In *Campylobacter*, ed. MJ Blaser, pp. 467–81. Washington, DC: ASM Press
68. Jacques MA, Josi K, Darrasse A, Samson R. 2005. *Xanthomonas axonopodis* pv. *phaseoli* var. fuscans is aggregated in stable biofilm population sizes in the phyllosphere of field-grown beans. *Appl. Environ. Microbiol.* 71:2008–15
69. Jaeger CH, Lindow SE, Miller S, Clark E, Firestone MK. 1999. Mapping of sugar and amino acid availability in soil around roots with bacterial sensors of sucrose and tryptophan. *Appl. Environ. Microbiol.* 65:2685–90
70. Janisiewicz WJ, Conway WS, Brown MW, Sapers GM, Fratamico P, Buchanan RL. 1999. Fate of *Escherichia coli* O157:H7 on fresh-cut apple tissue and its potential for transmission by fruit flies. *Appl. Environ. Microbiol.* 65:1–5
71. Jeter C, Matthysse AG. 2005. Characterization of the binding of diarrheagenic strains of *E. coli* to plant surfaces and the role of curli in the interaction of the bacteria with alfalfa sprouts. *Mol. Plant-Microbe Interact.* 18:1235–42
72. Jha AK, Bais HP, Vivanco JM. 2005. *Enterococcus faecalis* mammalian virulence-related factors exhibit potent pathogenicity in the *Arabidopsis thaliana* plant model. *Infect. Immun.* 73:464–75
73. Johnson KB, Stockwell VO, Sawyer TL, Sugar D. 2000. Assessment of environmental factors influencing growth and spread of *Pantoea agglomerans* on and among blossoms of pear and apple. *Phytopathology* 90:1285–94
74. Jurkevitch E, Hadar Y, Chen Y, Chino N. Mori S. 1993. Indirect utilization of the phytosiderophore mugineic acid as an iron source to rhizosphere fluorescent *Pseudomonas*. *BioMetals* 6:119–23
75. Kader A. 1996. In-plant storage. In *Almond Production Manual*, ed. W Micke. Oakland, CA: Div. Agric. Nat. Resourc., Univ. Calif.
76. Kim JJ, Sundin GW. 2000. Regulation of the *rulAB* mutagenic DNA repair operon of *Pseudomonas syringae* by UV-B (290 to 320 nanometers) radiation and analysis of *rulAB*-mediated mutability in vitro and in planta. *J. Bacteriol.* 182:6137–44
77. Kudva IT, Blanch K, Hovde CJ. 1998. Analysis of *Escherichia coli* O157:H7 survival in ovine or bovine manure and manure slurry. *Appl. Environ. Microbiol.* 64:3166–74
78. Kumar A, Agarwal RK, Bhilegaonkar KN, Shome BR, Bachhil VN. 2001. Occurrence of *Campylobacter jejuni* in vegetables. *Int. J. Food Microbiol.* 67:153–55
79. Leben C. 1988. Relative humidity and the survival of epiphytic bacteria with buds and leaves of cucumber plants. *Phytopathology* 78:179–85
80. Lee SW, Cooksey DA. 2000. Genes expressed in *Pseudomonas putida* during colonization of a plant-pathogenic fungus. *Appl. Environ. Microbiol.* 66:2764–72
81. Leveau JHJ, Lindow SE. 2001. Appetite of an epiphyte: quantitative monitoring of bacterial sugar consumption in the phyllosphere. *Proc. Natl. Acad. Sci. USA* 98:3446–53
82. Liao CH, Sapers GM. 1999. Influence of soft rot bacteria on growth of *Listeria monocytogenes* on potato tuber slices. *J. Food Prot.* 62:343–48
83. Lilley AK, Bailey MJ. 1997. The acquisition of indigenous plasmids by a genetically marked pseudomonad population colonizing the sugar beet phytosphere is related to local environment conditions. *Appl. Environ. Microbiol.* 63:1577–83
84. Lin E. 1996. Dissimilatory pathways for sugars, polyols, and carboxylates. See Ref. 109a, pp. 307–42
85. Lindow SE, Andersen G, Beattie GA. 1993. Characteristics of insertional mutants of *Pseudomonas syringae* with reduced epiphytic fitness. *Appl. Environ. Microbiol.* 59:1593–601

86. Lindow SE, Andersen GL. 1996. Influence of immigration on epiphytic bacterial populations on navel oranges. *Appl. Environ. Microbiol.* 62:2978–87
87. Lindow SE, Brandl MT. 2003. Microbiology of the phyllosphere. *Appl. Environ. Microbiol.* 69:1875–83
87a. Lindow SE, Hecht-Poinar EI, Elliot VJ, eds. 2002. *Phyllosphere Microbiology*. St. Paul, MN: APS Press
88. Loper JE, Henkels MD. 1999. Utilization of heterologous siderophores enhances levels of iron available to *Pseudomonas putida* in the rhizosphere. *Appl. Environ. Microbiol.* 65:5357–63
89. Lopez-Solanilla E, Garcia-Olmedo F, Rodriguez-Palenzuela P. 1998. Inactivation of the *sapA* to *sapF* locus of *Erwinia chrysanthemi* reveals common features in plant and animal bacterial pathogenesis. *Plant Cell* 10:917–24
90. Lopez-Solanilla E, Llama-Palacios A, Collmer A, Garcia-Olmedo F, Rodriguez-Palenzuela P. 2001. Relative effects on virulence of mutations in the *sap*, *pel*, and *hrp* loci of *Erwinia chrysanthemi*. *Mol. Plant-Microbe Interact.* 14:386–93
91. Lugtenberg BJ, Dekkers LC. 1999. What makes *Pseudomonas* bacteria rhizosphere competent? *Environ. Microbiol.* 1:9–13
92. Mandrell RE, Brandl MT. 2004. *Campylobacter* species and fresh produce: Outbreaks, incidence and biology. See Ref. 8a, pp. 59–72
93. Mandrell RE, Gorski L, Brandl MT. 2006. Attachment of microorgamisms to fresh produce. In *Microbiology of Fruits and Vegetables*, ed. GM Sapers, JR Gorny, AE Yousef, pp. 33–73. Boca Raton, FL: CRC Press
93a. Matthews KR, ed. 2006. *Microbiology of Fresh Produce*. Washington, DC: ASM Press
94. Mercier J, Lindow SE. 2000. Role of leaf surface sugars in colonization of plants by bacterial epiphytes. *Appl. Environ. Microbiol.* 66:369–74
95. Miller CD, Kim YC, Anderson AJ. 2001. Competitiveness in root colonization by *Pseudomonas putida* requires the *rpoS* gene. *Can. J. Microbiol.* 47:41–48
96. Miller WG, Brandl MT, Quinones B, Lindow SE. 2001. Biological sensor for sucrose availability: relative sensitivities of various reporter genes. *Appl. Environ. Microbiol.* 67:1308–17
97. Mitscherlich E, Marth EH. 1984. *Microbial Survival in the Environment*. Berlin: Springer Verlag

References 98–101 describe the role of aggregate formation in the population dynamics and survival of bacteria in the phyllosphere.

98. Monier JM, Lindow SE. 2003. Differential survival of solitary and aggregated bacterial cells promotes aggregate formation on leaf surfaces. *Proc. Natl. Acad. Sci. USA* 100:15977–82
99. Monier JM, Lindow SE. 2004. Frequency, size, and localization of bacterial aggregates on bean leaf surfaces. *Appl. Environ. Microbiol.* 70:346–55
100. Monier JM, Lindow SE. 2005. Aggregates of resident bacteria facilitate survival of immigrant bacteria on leaf surfaces. *Microb. Ecol.* 49:343–52
101. Monier JM, Lindow SE. 2005. Spatial organization of dual-species bacterial aggregates on leaf surfaces. *Appl. Environ. Microbiol.* 71:5484–93

The authors describe the first isolation of naturally occurring biofilms on the leaf surface of various vegetable crops.

102. Montville TJ, Matthews KR. 2005. *Food Microbiology, An Introduction*. Herdon, VA: ASM Press. 380 pp.
103. Morgan JV, Tukey HB. 1964. Characterization of leachate from plant foliage. *Plant Physiol.* 39:590–93
104. Morris CE, Monier JM, Jacques MA. 1997. Methods for observing microbial biofilms directly on leaf surfaces and recovering them for isolation of culturable microorganisms. *Appl. Environ. Microbiol.* 63:1570–76

105. Muller T, Ulrich A, Ott EM, Muller M. 2001. Identification of plant-associated enterococci. *J. Appl. Microbiol*. 91:268–78
106. Nachin L, Barras F. 2000. External pH: an environmental signal that helps to rationalize *pel* gene duplication in *Erwinia chrysanthemi*. *Mol. Plant-Microbe Interact*. 13:882–86
107. Naimi TS, Wicklund JH, Olsen SJ, Krause G, Wells JG, et al. 2003. Concurrent outbreaks of *Shigella sonnei* and enterotoxigenic *Escherichia coli* infections associated with parsley: implications for surveillance and control of foodborne illness. *J. Food Prot.* 66:535–41
108. Natl. Inst. Health Infect. Dis. Control Div. 1998. Enterohemorrhagic *Escherichia coli* (verocytotoxin-producing *E. coli*) infection, 1996—April 1998. *Infect. Agents Surveill. Rep*. 19:122–23
109. Natvig EE, Ingham SC, Ingham BH, Cooperband LR, Roper TR. 2002. *Salmonella enterica* serovar Typhimurium and *Escherichia coli* contamination of root and leaf vegetables grown in soils with incorporated bovine manure. *Appl. Environ. Microbiol*. 68:2737–44
109a. Neidhardt F, ed. 1996. *Escherichia coli and Salmonella: Cellular and Molecular Biology*. Washington, DC: ASM Press
110. Nelson EB, Chao WL, Norton JM, Nash GT, Harman GE. 1986. Attachment of *Enterobacter cloacae* to hyphae of *Pythium ultimum*: possible role in the biological control of *Pythium* preemergence damping-off. *Phytopathology* 76:327–35
111. Nohmi T, Hakura A, Nakai Y, Watanabe M, Murayama SY, Sofuni T. 1991. *Salmonella typhimurium* has two homologous but different *umuDC* operons: cloning of a new *umuDC*-like operon (*samAB*) present in a 60-megadalton cryptic plasmid of *S. typhimurium*. *J. Bacteriol*. 173:1051–63
112. Normander B, Christensen BB, Molin S, Kroer N. 1998. Effect of bacterial distribution and activity on conjugal gene transfer on the phylloplane of the bush bean (*Phaseolus vulgaris*). *Appl. Environ. Microbiol*. 64:1902–9
113. O'Brien RD, Lindow SE. 1989. Effect of plant species and environmental conditions on epiphytic population sizes of *Pseudomonas syringae* and other bacteria. *Phytopathology* 79:619–27
114. Park CE, Sanders GW. 1992. Occurrence of thermotolerant campylobacters in fresh vegetables sold at farmers' outdoor markets and supermarkets. *Can. J. Microbiol*. 38:313–16
115. Parke JL, Gurian-Sherman D. 2001. Diversity of the *Burkholderia cepacia* complex and implications for risk assessment of biological control strains. *Annu. Rev. Phytopathol*. 39:225–58
116. Parra-Lopez C, Baer MT, Groisman EA. 1993. Molecular genetic analysis of a locus required for resistance to antimicrobial pertides in *Salmonella* Typhimurium. *EMBO J*. 12:4053–62
117. Payne SM. 1988. Iron and virulence in the family Enterobacteriaceae. *Crit. Rep. Microbiol*. 16:81–111
118. Poza-Carrion C, Lindow SE. 2004. Influence of indigenous bacteria on survival of human pathogens on plants. *Phytopathology* 94(Suppl.): 128
119. Rahme LG, Stevens EJ, Wolfort SF, Shao J, Tompkins RG, Ausubel FM. 1995. Common virulence factors for bacterial pathogenicity in plants and animals. *Science* 268:1899–902
120. Ramos C, Molbak L, Molin S. 2000. Bacterial activity in the rhizosphere analyzed at the single-cell level by monitoring ribosome contents and synthesis rates. *Appl. Environ. Microbiol*. 66:801–9

This comparative study provides the first quantitative evidence for differences in epiphytic fitness between leaf-associated bacteria and human pathogens.

The authors provide molecular evidence that *P. aeruginosa* uses common strategies to interact with their plant and human hosts. This work created a shift of paradigm about pathogen-host relationships.

121. Rangel JM, Sparling PH, Crowe C, Griffin PM, Swerdlow DL. 2005. Epidemiology of *Escherichia coli* O157:H7 outbreaks, United States, 1982–2002. *Emerg. Infect. Dis.* 11:603–9
122. Rasch M, Andersen JB, Nielsen KF, Flodgaard LR, Christensen H, et al. 2005. Involvement of bacterial quorum-sensing signals in spoilage of bean sprouts. *Appl. Environ. Microbiol.* 71:3321–30
123. Rayner J, Veeh R, Flood J. 2004. Prevalence of microbial biofilms on selected fresh produce and household surfaces. *Int. J. Food Microbiol.* 95:29–39
124. Richards GM, Beuchat LR. 2005. Infection of cantaloupe rind with *Cladosporium cladosporioides* and *Penicillium expansum*, and associated migration of *Salmonella poona* into edible tissues. *Int. J. Food Microbiol.* 103:1–10
125. Riordan DC, Sapers GM, Annous BA. 2000. The survival of *Escherichia coli* O157:H7 in the presence of *Penicillium expansum* and *Glomerella cingulata* in wounds on apple surfaces. *J. Food Prot.* 63:1637–42
126. Rojas CM, Ham JH, Deng WL, Doyle JJ, Collmer A. 2002. HecA, a member of a class of adhesins produced by diverse pathogenic bacteria, contributes to the attachment, aggregation, epidermal cell killing, and virulence phenotypes of *Erwinia chrysanthemi* EC16 on *Nicotiana clevelandii* seedlings. *Proc. Natl. Acad. Sci. USA* 99:13142–47
127. Romantschuk M. 2004. Bacterial attachment to leaves. In *Encyclopedia of Plant and Crop Science*, ed. RM Goodman, pp. 75–78. New York: Marcel Dekker
128. Romling U. 2002. Molecular biology of cellulose production in bacteria. *Res. Microbiol.* 153:205–12
129. Sabaratnam S, Beattie GA. 2003. Differences between *Pseudomonas syringae* pv. *syringae* B728a and *Pantoea agglomerans* BRT98 in epiphytic and endophytic colonization of leaves. *Appl. Environ. Microbiol.* 69:1220–28
130. Sasaki T, Kobayashi M, Agui N. 2000. Epidemiological potential of excretion and regurgitation by *Musca domestica* (Diptera: Muscidae) in the dissemination of *Escherichia coli* O157:H7 to food. *J. Med. Entomol.* 37:945–49
131. Savageau MA. 1983. *Escherichia coli* habitats, cell types, and molecular mechanisms of gene control. *Am. Nat.* 122:732–44
132. Schauder S, Bassler BL. 2001. The languages of bacteria. *Genes Dev.* 15:1468–80
133. Schlech WF, Lavigne PM, Bortolussi RA, Allen AC, Haldane EV, et al. 1983. Epidemic listeriosis—evidence for transmission by food. *N. Engl. J. Med.* 308:203–6
134. Sela S, Nestel D, Pinto R, Nemny-Lavy E, Bar-Joseph M. 2005. Mediterranean fruit fly as a potential vector of bacterial pathogens. *Appl. Environ. Microbiol.* 71:4052–56
135. Sivapalasingam S, Friedman CR, Cohen L, Tauxe RV. 2004. Fresh produce: a growing cause of outbreaks of foodborne illness in the United States, 1973 through 1997. *J. Food Prot.* 67:2342–53
136. Solomon EB, Brandl MT, Mandrell RE. 2006. Biology of food-borne pathogens on produce. See Ref. 93a, pp. 55–83
137. Solomon EB, Pang HJ, Matthews KR. 2003. Persistence of *Escherichia coli* O157:H7 on lettuce plants following spray irrigation with contaminated water. *J. Food Prot.* 66:2198–202
138. Starr MP, Chatterjee AK. 1972. The genus *Erwinia*: enterobacteria pathogenic to plants and animals. *Annu. Rev. Microbiol.* 26:389–426
139. Staskawicz BJ, Mudgett MB, Dangl JL, Galan JE. 2001. Common and contrasting themes of plant and animal diseases. *Science* 292:2285–89
140. Stockwell VO, Loper JE. 2005. The sigma factor RpoS is required for stress tolerance and environmental fitness of *Pseudomonas fluorescens* Pf-5. *Microbiology* 151:3001–9

141. Sukupolvi S, Lorenz RG, Gordon JI, Bian Z, Pfeifer JD, et al. 1997. Expression of thin aggregative fimbriae promotes interaction of *Salmonella* Typhimurium SR-11 with mouse small intestinal epithelial cells. *Infect. Immun.* 65:5320–25
142. Sundin GW. 2002. Ultraviolet radiation on leaves: its influence on microbial communities and their adaptations. See Ref. 87a, pp. 27–41
143. Sundin GW, Murillo J. 1999. Functional analysis of the *Pseudomonas syringae rulAB* determinant in tolerance to UV B (290–320 nm) radiation and distribution of *rulAB* among *P. syringae* pathovars. *Environ. Microbiol.* 1:75–87
144. Surette MG, Bassler BL. 1999. Regulation of autoinducer production in *Salmonella typhimurium*. *Mol. Microbiol.* 31:585–95
145. Takeuchi K, Frank JF. 2001. Expression of red-shifted green fluorescent protein by *Escherichia coli* O157:H7 as a marker for the detection of cells on fresh produce. *J. Food Prot.* 64:298–304
146. Takeuchi K, Matute CM, Hassan AN, Frank JF. 2000. Comparison of the attachment of *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella typhimurium*, and *Pseudomonas fluorescens* to lettuce leaves. *J. Food Prot.* 63:1433–37
147. Takkinen J, Nakari UM, Johansson T, Niskanen T, Siitonen A, Kuusi M. 2005. A nationwide outbreak of multiresistant *Salmonella* Typhimurium va. Copenhagen DT104B infection in Finland due to contaminated lettuce from Spain. *Eurosurveill. Wkly.* **http://www.eurosurveillance.org**
148. Tauxe R, Kruse H, Hedberg C, Potter M, Madden J, Wachsmuth K. 1997. Microbial hazards and emerging issues associated with produce; a preliminary report to the national advisory committee on microbiologic criteria for foods. *J. Food Prot.* 60:1400–8
149. Tauxe RV. 1997. Emerging foodborne diseases: an evolving public health challenge. *Emerg. Infect. Dis.* 3:425–34
150. Torres AG, Jeter C, Langley W, Matthysse AG. 2005. Differential binding of *Escherichia coli* O157:H7 to alfalfa, human epithelial cells, and plastic is mediated by a variety of surface structures. *Appl. Environ. Microbiol.* 71:8008–15
151. Tsang THF, Brawley L, Abbott S, Werner SB, Vugia D. 1999. Large *Shigella sonnei* outbreak linked to salsa in California. In *Abstr. Epidemic Intell. Serv. Conf., 48th*. US Cent. Dis. Prev. Control, Atlanta
152. Uesugi AR, Harris LJ. 2006. Growth of *Salmonella* Enteritidis phage Type 30 in almond hull and shell slurries and survival in drying almonds hulls. *J. Food Prot.* 69: 712–18
153. Uhlich GA, Keen JE, Elder RO. 2002. Variations in the *csgD* promoter of *Escherichia coli* O157:H7 associated with increased virulence in mice and increased invasion of HEp-2 cells. *Infect. Immun.* 70:395–99
154. Ukuku DO, Fett WF. 2002. Relationship of cell surface charge and hydrophobicity to strength of attachment of bacteria to cantaloupe rind. *J. Food Prot.* 65:1093–99
155. US Cent. Dis. Control Prev. 1994. Foodborne outbreaks of enterotoxigenic *Escherichia coli*—Rhode Island and New Hampshire, 1993. *MMWR* 43:87–89
156. US Cent. Dis. Control Prev. 2003. 2002—Foodborne disease outbreaks due to bacterial etiologies. **http://www.cdc.gov/foodborneoutbreaks/us_outb/fbo2002./bacterial02.htm**
157. US Cent. Dis. Control Prev. 2004. Foodborne outbreaks due to bacterial etiologies, 2003. **http://www.cdc.gov/foodborneoutbreaks/us_outb/fbo2003./summary03.htm**
158. US Cent. Dis. Control Prev. 2004. Outbreaks of *Salmonella* serotype Enteritidis infections associated with raw almonds—United States and Canada, 2003–2004. *MMWR* 53:1–3

159. US Cent. Dis. Control Prev. 2005. Foodborne outbreaks due to bacterial etiologies. 2004. **http://www.cdc.gov/foodborneoutbreaks/us_outb/fbo2004./summary04.htm**
160. US Dep. Agric. 2004. Progress update and 2002 data summary. Microbial Data Program. **http://www.ams.usda.gov/science/mpo/MDPSumm02.pdf**
161. US Food Drug Admin. 2001. FDA survey of imported fresh produce. Cent. Food Saf. Appl. Nutr. **http://www.cfsan.fda.gov/~dms/prodsur6.html**
162. US Food Drug Admin. 2001. Survey of domestic fresh produce. Cent. Food Saf. Appl. Nutr. **http://www.cfsan.fda.gov/~dms/prodsur9.html**
163. Vibha V, Ailes E, Wolyniak C, Angulo F, Klontz K. 2006. Recalls of spices due to bacterial contamination monitored by the U.S. Food and Drug Administration: the predominance of Salmonellae. *J. Food Prot*. 69:233–37
164. Von Bodman SB, Bauer WD, Coplin DL. 2003. Quorum sensing in plant-pathogenic bacteria. *Annu. Rev. Phytopathol*. 41:455–82
165. Wade WN, Beuchat LR. 2003. Metabiosis of proteolytic moulds and *Salmonella* in raw, ripe tomatoes. *J. Appl. Microbiol*. 95:437–50
166. Wade WN, Vasdinnyei R, Deak T, Beuchat LR. 2003. Proteolytic yeasts isolated from raw, ripe tomatoes and metabiotic association of *Geotrichum candidum* with *Salmonella*. *Int. J. Food Microbiol*. 86:101–11
167. Watanabe Y, Ozasa K, Mermin JH, Griffin PM, Masuda K, et al. 1999. Factory outbreak of *Escherichia coli* O157:H7 infection in Japan. *Emerg. Infect. Dis.* 5:424–28
168. Wells JM, Butterfield JE. 1997. *Salmonella* contamination associated with bacterial soft-rot of fresh fruits and vegetables in the marketplace. *Plant Dis*. 81:867–72
169. Wilson M, Hirano SS, Lindow SE. 1999. Location and survival of leaf-associated bacteria in relation to pathogenicity and potential for growth within the leaf. *Appl. Environ. Microbiol*. 65:1435–43
170. Wilson M, Lindow SE. 1994. Coexistence among epiphytic bacterial populations mediated through nutritional resource partitioning. *Appl. Environ. Microbiol*. 60:4468–77
171. Wilson M, Lindow SE. 1994. Ecological similarity and coexistence of epiphytic ice-nucleating (Ice^+) *Pseudomonas syringae* strains and a nonice-nucleating (Ice^-) biological control agent. *Appl. Environ. Microbiol*. 60:3128–37
172. Woodgate R, Sedgwick SG. 1992. Mutagenesis induced by bacterial UmuDC proteins and their plasmid homologues. *Mol. Microbiol*. 6:2213–18
173. WHO. 2000. *Seventh report of WHO surveillance program for control of foodborne infections and intoxications in Europe.* FAO/WHO Collab. Cent. Res. Train. Food Hygiene Zoonoses
174. WHO. 2003. *Eighth report of WHO surveillance program for control of foodborne infections and intoxications in Europe*. Collab. Cent. Res. Train. Food Hygiene Zoonoses
175. Wu FM, Doyle MP, Beuchat LR, Wells JG, Mintz ED, Swaminathan B. 2000. Fate of *Shigella sonnei* on parsley and methods of disinfection. *J. Food Prot*. 63:568–72
176. Yang CH, Menge JA, Cooksey DA. 1994. Mutations affecting hyphal colonization and pyoverdine production in Pseudomonads antagonistic toward *Phytophthora parasitica*. *Appl. Environ. Microbiol*. 60:473–81

This study is the foundation of several investigations of the interaction of enteric pathogens with plant pathogens on produce.