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Alternative Ecology of Human Pathogenic Bacteria in Fruits and Vegetables

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Abstract: Outbreaks of illness due to human enteric pathogenic bacteria via fresh vegetables warrant intensive research on changing strategies of these bacteria in alterning their hosts for survival. The systemic infection of human pathogenic bacteria in plants and the plant growth stage at which they establish endophytic relationship is poorly understood. The issue is magnified in countries like India where the dietary habits are changing and consumption of fresh fruits and vegetables as salad has become a part in the everyday menu of most people. Most of the human pathogenic enteric bacteria are generally characterized by broad host ranges and these pathogens seem to exploit almost any change in human ecology that provides new opportunities for transmission. Because plants are not traditionally considered as hosts for human enteric pathogens, recent produce-associated outbreaks highlight important deficiencies in our understanding of the ecology of enteric pathogens outside of their human and animal hosts. This review focuses on understanding the human enteric pathogens that have developed abilities to colonize internal tissues of vegetables and fruits popularly consumed as salads, how and when do they enter plants and where do they localize in plant tissues. In addition, we have also highlighted the attempts made in detection and control of these bacteria in plant hosts. This understanding will help develop strategies towards vegetable food safety in a joint effort by agriculturalists, environmentalists, food processing agencies, whole salers and retailers, which will ultimately benefit every consumer.

Key words: Bacteria, enteropathogenic, fruits, human, pathogenic, vegetables

INTRODUCTION

Food safety has become a major issue in the agro-food chain in the last decades. Foods can be contaminated by physical, chemical and biological ways, of which the impact of biological hazards due to plant and human pathogenic microbes have great concern among the public than the other hazards (Rovira *et al*., 2006). This is probably because biological risks are more frequently reported, can affect a large number of consumers and generally induce acute symptoms. The major reservoir of these pathogens consists of (healthy) agricultural animals and irrigation water used in agriculture, from which they spread to an increasingly variety of agricultural produce like vegetables and fruits. Traditionally, these pathogens were primarily associated with food products of animal origin like meat and eggs. However, recent outbreaks of food borne disease associated with the consumption of fresh produce, especially vegetables raised concern that these products may be an increasing source of food borne infections caused by alimentary system pathogens.

In recent years, the awareness of food poisoning by human pathogens in fresh vegetables has increased, as shown by a strong increase of reported incidents for *E. coli* O157:H7 and *Salmonella* spp, among many other bacteria which are sparsely reported. Most outbreaks related to consumption of fresh or unprocessed vegetables are reported for *E. coli* O157:H7 and *Salmonella* spp. In addition to the fresh and unprocessed vegetables, the minimally processed fruits and vegetables also have been reported to contribute to the food-borne infection significantly (De Roever, 1998). The recognition of fresh fruits and vegetables as major vehicles of food borne illness has led to increased research on mechanisms by which these human pathogens contaminate and persists on and in this non-host environment. Interactions between food borne pathogens and plants as well among the naturally occurring microbial communities contribute to endophytic and epiphytic colonization. Scientific findings are just beginning to find the mechanisms that contribute to colonization of pathogens (Critzer and Doyle, 2010).

This review is to update the current knowledge of the human pathogenic bacteria in fruits and vegetables, their infection strategies and changing ecology as well as to explore the further research needed to increase our understanding of the microbial ecology of enteric pathogens on fruits and vegetables so that combating designs could be arrived.

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Sources of contamination: Until now, it is not clear what the actual risks are for introducing health threatening organisms like *E. coli* O157:H7 and *Salmonella* spp in the production chain and how they will affect the safety of the product for the consumer. In the farm to table production, processing and distribution of fruits and vegetables, there are various possible points of contamination with disease causing microorganisms. These include irrigation water, manure, wastewater, handling by workers and contact with contaminated surfaces. Main source of contamination of fresh fruits and vegetables are through contaminated water, feces, contaminated manure or compost, contaminated soil, insects, contaminated seeds as reported by Critzer and Doyle (2010). In many developing countries, waste water use in agriculture is common practice and is increasing as a result of the rising water scarcity worldwide (Scott *et al*., 2004). Human enteric bacteria arrive at the agricultural soils from the excretion of animals and sewage water irrigation. Same strains of entero-pathogenic *E. coli* and *Salmonella* present in edible parts of vegetables were isolated in the contaminated raw wastewater used to irrigate the plants (Ibenyassine *et al*., 2007). In addition to the irrigation of the agricultural fields directly with waste water, runoff from contaminated areas such as cow pastures also are reported to spread bacteria to the lands where crops are cultivated (Muirhead *et al*., 2006). Beuchat (2002) reports that the manure used as a fertilizer or soil amendment as the potential source of pathogenic bacteria to contaminate fruits and vegetables. Yet another possible mechanism for this transfer is through nematodes, *Caenorhabditis elegans*, which are capable of transporting *Salmonella* to fruits and vegetables through soil (Beuchat, 2002).

Human enteric bacteria in produce: There are a number of reports indicating that raw vegetables may harbour potential foodborne pathogens (Nguyen-The and Carlin, 2000). Initially, *Listeria monocytogenes* (Schlech *et al*., 1983), *Salmonella* (Doyle, 1990) and *E. coli* (Nguyen-The and Carlin, 2000) were reported from raw vegetables. In recent years, as the traditional routes of infection are better controlled, large outbreaks of nontyphoidal *Salmonella* infection have been attributed to fruits, vegetables and processed foods (Andrews-Polymenis *et al*., 2009). *Salmonella* and enterovirulent *E. coli* are capable of spending at least a part of their life cycle as plant-associated endo or epiphytes. *Salmonella* Typhimurium has been demonstrated to use plants as alternate hosts (Schikora *et al*., 2012).

It has been demonstrated that *S. typhimurium* overcomes the innate immune response of *Arabidopsis thaliana* and shows an endopathogenic lifestyle (Schikora *et al*., 2012). Guo *et al*. (2001) reported that tomato stems and flowers are possible sites at which *Salmonella* may attach and remain viable during fruit development, thus serving as routes or reservoirs for contaminating ripened fruit. Lettuce plants grown in soil amended with contaminated irrigation water or manure slurry became internally contaminated in the edible part under conditions where external contamination of the edible part was prevented. Members of the brassicaceae family (radish, turnip and broccoli) had the highest prevalence of *Salmonella* contamination than lettuce, tomato and carrots when sown and grown in contaminated soil (Barak *et al*., 2008).

The key virulence factor produced by *E.coli* 0157:H7 is Shiga toxin, which is known to be carried by several different phages with relatively indiscriminant host ranges. Recent investigations have demonstrated that the phage is mobilized under specific conditions, the same conditions that increase production of toxin itself (Zhang *et al*., 2000). Janisiewicz *et al*. (1999) demonstrated that fruit flies contaminated with a fluorescent-tagged nonpathogenic strain of *E. coli* O157:H7 served as a vector in colonization of the organism in apple wounds.

Staphylococcus aureus is the species most commonly associated with staphylococcal food poisoning. It is usually found in ready to eat foods. Contamination frequently occurs through improper handling and between up to 30 and 50% of the human population carries *S. aureus* as commensals (Le Loir *et al*., 2003). Several outbreaks of salmonellosis have been associated with the consumption of cut cantaloupe and watermelon. In 1991 a *Salmonella javiana* outbreak among school children revealed a strong association with the consumption of watermelon (Blostein, 1993). More than 400 laboratory confirmed infections with *Salmonella* occurred in 23 USA states and in Canada during June and July 1991, related to the consumption of cantaloupes.

Listeria monocytogenes is widely distributed in the environment, where it is associated with decaying vegetables, soil, sewage and feces of animals and has been isolated from several types of vegetables (Beuchat, 1996). Cases of human listeriosis that have been associated with the consumption of raw vegetables are likely, in part, due to contamination by manure from ruminants. It is known to grow on a variety of vegetables at refrigeration temperatures. *Aeromonas* spp. isolated from vegetables have been shown to represent a potential risk to consumers health (Pedroso *et al*., 1997). We have recently reported the occurrence of *Staphylococcus warneri* in fresh marketed apples (Phukon *et al*., 2013).

Colonization and infection: Attachment of enteric pathogens is the first step towards colonization of the non host plant environment. Research on the mechanisms

of attachment by food borne pathogens has revealed fimbriae, flagella and biofilms (Critzer and Doyle, 2010). Evidence for the host regulation of endophytic colonization by enteric bacteria came from the observation that the interior of an ethylene-insensitive mutant of *Medicago truncatula* was colonized in much higher numbers than the wild-type plant (Iniguez *et al*., 2005).

Enteric pathogens are found to be present inside plant seedling and are found in the intracellular spaces between host cell walls and causes typical entophytic infection (Dong *et al*., 2003). Endophytic colonization of human pathogens in the plants has been observed in several studies, but how the bacteria enter these plants and where they are localized within the plants are incompletely understood. Endophytic colonization varies according to the bacterium and plant species examined. From numerous observations of bacterial colonization of plant roots, it is widely assumed that bacteria can colonize and enter roots at sites of lateral root (Tyler and Triplett, 2008). Confocal microscopy of the entophyte *K. pneumoniae* 342 on plants revealed significant colonization around lateral root cracks of *M. sativa*, *M. truncatula*, *A. thaliana*, *Triticum aestivum* and *Oryza sativa*, suggesting a possible entry site for the bacteria into the plant (Dong *et al*., 2003).

Endophytic colonization of *E. coli* 0157:H7 with in fruits and vegetables has been studied and has been observed to colonize apple lenticles, to a depth of 40 µl meters and in core and subsurface structures attaching to the cartilaginous pericarp and seed integuments with infiltration occurring through the blossom's calyx and traveling up the floral tube to the internal parts of the apple (Burnett *et al*., 2000). *E. coli* 0157:H7 was observed to colonize and survive by causing cell membranes to degrade and release their contents and with on apple tissues forms granules and vesicles due to osmotic conditions in the apple tissues (Janes *et al*., 2005).

In orange, *E. coli* 0157:H7 and *Salmonella* colonize the interior of fruits by passive mechanism of entry (Eblen *et al*., 2004). *E. coli* found in root-inoculated radish sprouts was observed within stomata of cotyledons and the inner tissues of edible hypocotyls and cotyledons. In carrots, *E. coli* was found at cell junctions and intercellular spaces up to 50 ìm within carrot tissue, but did not penetrate the carrot cells (Auty *et al*., 2005). *E. coli* O157:H7 was observed colonizing preferentially at root junctions on lettuce (Jablasone *et al*., 2005).

The roots and xylem of citrus plants can also be colonized by enteric bacteria. *K. pneumonia* 342 (Kp342) was able to endophytically colonize both *Citrus sinensis* and *Catharanthus roseus*, though it was present in higher numbers in *C. roseus* plants. Kp342 has also been found to colonize the interiors of *Medicago sativa* (alfalfa), *M. truncatula*, *A. thaliana*, *Tritium aestivum* (wheat) and *Oryza sativa* (Dong *et al*., 2003). *Salmonella* mutants deficient in the production of flagella or one of the type III secretion systems showed increased endophytic colonization (Iniguez *et al*., 2005).

Pseudomonas putida KT2440, a TPS, *hlpBA*, is involved in seed colonization and iron uptake. The *hlpA* gene encodes a secreted protein similar to iron-regulated hemolysins and HlpB is responsible for its transport across the membrane (Molina *et al*., 2006). The molecular mechanisms used by bacteria to colonize the interior of plants are still largely unknown (Dong *et al*., 2003). Most studies focus on reporting the internalization of bacteria and their general location on and within plants, though some have linked bacterial colonization to specific genes. While many studies have been done on plant growth promoting bacteria or plant pathogens, similar colonization mechanisms may be utilized by human pathogens (Iniguez *et al*., 2005). Microarray analysis revealed that several pathogenicity genes, including type II secretion genes and a gene involved in capsular polysaccharide synthesis, were induced in *S. enterica* serovar Typhimurium during colonization in response to lettuce root exudates (Klerks *et al*., 2007).

Plant–human bacteria interaction: Molecular level interaction between human pathogenic bacteria and the plants is thought to play a crucial role in infection and colonization of the plant. It is now realized that the presence of an active and controlled interaction between pathogen and plant is based on presence or absence of certain genes/proteins in the plant and/or pathogen. Many of the interactions that occur between food-borne pathogens and the fruits and vegetables they contaminate are just beginning to be elucidated. There are several factors, such as produce type, cultivar and physiological state of the plants and pathogen that influence the colonization of food borne pathogens on produce (Critzer and Doyle, 2010).

Colonization of *S. typhimurium* on the plant seedlings was partially dependent on avoiding both salicylic acid (SA)-dependent and SA- independent plant defenses, inducible defenses that are often triggered by flagella and components of the TTSS (Iniguez *et al*., 2005). Plant colonization by an endophytic *K. pneumoniae* was also limited by the induced systemic resistance, but *Klebsiella* manages to avoid plant's SA-dependent defenses (Iniguez *et al*., 2005). This finding suggests that *Salmonella* can be recognized by plants and that general host defenses can be induced. However, it is not yet clear at which threshold population densities enteric pathogens will induce plant defenses in planta during natural interactions.

Differences in the interactions of *Salmonella* serovars with host plant varieties (Barak *et al*., 2008) further support a hypothesis that these interactions are genetically programmed. The identification of crop cultivars that may be less susceptible to colonization by human pathogens (Barak *et al*., 2008) would offer an economical way to reduce produce contamination. It is not yet known how *Salmonella* finds lesions formed by plant pathogens. It is possible that enterics recognize signals produced by plant pathogens (Brandl, 2006). For example, in *Salmonella* and *E. coli* a LuxR-type protein *SdiA* detects Acyl-Homoserine Lactone (AHL) Quorum Sensing (QS) signals, which are chemically similar to those produced by many soft rotting bacteria (Ahmer *et al.,* 2007). In response to the AHL signals, *Salmonella* SdiA induces expression of attachment genes (Smith and Ahmer, 2003). While plausible, these interactions have not yet been demonstrated in planta. This research will be facilitated by a recent development of sensitive tools for documenting sdia-mediated gene regulation in vivo (Ahmer *et al.,* 2007).

E. coli O157:H7 utilizes some cellular mechanisms to promote survival in acidic environments (Foster, 2000). One is an rpoS-dependent system, which is associated with the production of acid-shock proteins. The other is an rpoS-independent system, which removes excessive protons from the cytoplasm using the amino acid decarboxylase system. Changes in membrane fluidity can help protect bacterial cells from acidic environments as well (Yuk and Marshall, 2005). Price *et al.* (2004) reported that the rpoS dependent system is more important for survival of *E. coli* O157:H7 in apple cider but that the amino acid decarboxylase system is required for its survival in the bovine gastrointestinal tract.

There are several virulent genes produced by pathogens to infect plants such as *toxA, plcS*, *dbsA*, *hrpM*, *gacA* and *gacs* (Rahme *et al*., 2000). The *dsbA* gene encodes a periplasmic disulfide bond-forming enzyme and may function to affect periplasmic virulence-related proteins (Rahme *et al*., 2000). *DsbA* has been observed to be important to the pathogenicity of both human and plant pathogens, including *Shigella flexneri*, *Vibrio cholera* and *Erwinia chrysanthemi* (Watarai *et al*., 1995).

The *hrp*M gene is homologous to the *E. coli* gene, *mdo*H, involved in membrane-derived oligosaccharide synthesis (Loubens *et al*., 1993). Whereas *Gacs* is the sensor kinase and *Gac*A gene functions as a transcriptional regulator of pathogenicity, gene encoding extracellular products (Rich *et al*., 1994). Specific genes such as *agfB*, *agfD* and *rpo*S are involved in production and regulation of curli and cellulose (Barak *et al*., 2005) are involved in colonization of pathogens in plants. The pathogenicity of verocytotoxin- producing *E. coli* is determined by the presence of several virulence factors, one of the key virulence factor is to produce shiga-toxins (stx) which consist of two types:stx1 and stx2, these genes are encoded on bacteriophage genomes integrates into *E. coli* and other enterobacteriaceae (Herold *et al*., 2004). *E. coli* 0157:H7 does not produce enzymes that can degrade plant polysaccharides (cellulose, hemicelluloses and starch), but can utilize soluble carbohydrates (like maltose) which are produced by starch degrading bacteria (Russell *et al*., 2000).

Approaches for detection and control: Rapid and specific methods to detect various pathogens, in fresh fruits and vegetables can be done by PCR, it is a flexible detection method that allows setting up common reaction conditions to detect multiple pathogens at once (Bhagwat, 2003) or even simultaneous detection in a single tube reaction of two or more pathogens by multiplex PCR (Park *et al*., 2006). Multiplex PCR has the potential of saving time and effort in the laboratory, thus lowering testing related costs in food industry (Perry *et al*., 2007). Culture based method is the traditional method for detecting pathogens in food, these method is limited by their poor sensitivity, specificity and it is also a time consuming method (Elizaquivel and Aznar, 2008).

It is well known that disinfection is one of the most critical processing steps in fresh cut vegetable production, affecting the quality safety and shelf life of the end product (Gil *et al*., 2009). The fresh cut industry had used chlorine as one of the most effective sanitizers to assure the safety of their produce. However, there is a trend in eliminating chlorine from the disinfection process because of the concerns about its efficacy on the produce and about the environmental and health risks associated with the formation of carcinogenic halogenated disinfection by products (Olmez and Kretzschmar, 2009).

Physical methods are more effective in removing bacteria from plant surfaces by use of shear force. Physical method including modern aeration, ultra sound, high pressure, high intensity electric field pulses (HELP), ultraviolet radiation, radiofrequency, ionizing radiation, irradiation such as gamma rays, electron beam or X-rays. US Food and Drug administration in 2008 gave its approval to use irradiation for killing pathogens on ice berg lettuce and spinach (Gil *et al*., 2009). Chemical methods of cleaning and sanitizing produce surfaces usually involve the application of mechanical washing in the presence of sanitizers, followed by rinsing with portable water (Artes and Allende, 2005).

Future prospects and conclution: Endophytic colonization of human pathogens into plants has been observed in several studies, but how the bacteria enter these plants and where they are localized within plants are incompletely understood. The internalization of human pathogens into produce is of particular interest since it may protect these bacteria from sanitizing treatments meant to make fruits and vegetables safe for consumption. Nevertheless, these outbreaks are well publicized and cause considerable alarm. Any outbreak stemming from raw produce can cause far more harm if not reported quickly and followed by rapid source identification. Plant derived outbreaks can be caused by many factors: Pathogens may be located on the plant surface or within plant tissues; pathogens may be unwittingly added to raw produce during food preparation. What is known about the ability of enteric bacteria to enter plants? To what extent do plant hosts regulate their entry? How do clinical enteric pathogens differ from plant-derived enteric pathogens? What do we still need to know to address this problem? How can improved knowledge lead to a reduction in outbreaks?

When colonizing plants, human pathogens undoubtedly come into contact with native bacteria and must overcome microbial competition to establish themselves. These interactions vary according to which pathogens and host plants are being studied and which bacteria are used as antagonists. The molecular mechanisms used by bacteria to colonize the interior of plants are still largely unknown. Observations that different bacteria colonize different plants to varying degrees indicate that colonization of the plant interior is an active process that is controlled by genetic determinants on both sides of the interaction. Most studies focus on reporting the internalization of bacteria and their general location on and within plants, though some have linked bacterial colonization to specific genes. While many studies have been done on plant growth promoting bacteria or plant pathogens, similar colonization mechanisms may be utilized by human pathogens.

A general lack of efficacy of sanitizers in removing or killing pathogens on raw fruits and vegetables has been attributed, in part, to their inaccessibility to locations within structures and tissues that may harbor pathogens. Understanding the ecology of pathogens and naturally occurring microorganisms is essential before interventions for elimination or control of growth can be devised.

Comparison of healthy plant gene-expression profiles with infected plant profiles, might show differences in expression patterns and identify the genes involved with the plant response of colonization by the human pathogen. Also, a study of genes expressed by the pathogen when infecting the plant, will contribute to insight in the infection mechanism used by the pathogen and may lead to a prediction of the risk of plant invasion based on the genetic make-up of plant and pathogen strain. Thus, investigating the interaction of the pathogen with the plant might elucidate the mechanism behind infection and colonization, subsequently leading to a better understanding of plant infection by human pathogens. It will give rise to suggestions for preventing contaminations of freshly consumed vegetables, reducing the health threat for consumers.

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