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Fresh produce as a potential vector for bacterial human pathogens

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The predicted massive growth in the world overall population and the proportion of the elderly together with climate change and water shortages pose major global challenges in ensuring food security, both in terms of availability and safety. The geographic and demographic changes are likely to force unprecedented changes in land usage and agricultural practices (i.e. bringing closer together animal and crop production and the need to recycle water), which might increase the risk of contamination. Fresh produce, particularly salad leaves that are consumed raw, is becoming an increasingly important source of human infections. Despite the increasing risk to public health little is currently known about the mechanism though which human pathogens bind to leaf surfaces or the plant traits that facilitate bacterial attachment.

Enteric pathogens such as enterohaemorrhagic *E. coli* (EHEC) O157:H7 and *Salmonella enterica,* which are ranked as key food-borne pathogens of humans, are often attributed to consumption or handling of contaminated bovine or chicken products. However, recent outbreaks of food poisoning have been associated with consumption of contaminated vegetable or salad produce and evidence suggests that such outbreaks are increasing (reviewed in Holden *et al*., 2009; Little and Gillespie 2008). Examples of international outbreaks of *S. enterica* and EHEC O157 linked to ready-to-eat plant produce include a Scandinavian/UK outbreak of *S.* Thompson infection associated with consumption of rocket leaves; a Danish outbreak of *S.* Anatum infection linked to imported basil an outbreak of *S.* Typhimurium DT204b in several European countries associated with consumption of lettuce and an outbreak of *S.* Senftenberg infection associated with imported Israeli basil affecting the UK, Denmark, the Netherlands and the USA (Pezzoli *et al*., 2008). The largest EHEC O157 outbreak to date, in 1996 in Sakai City, Osaka, Japan, was traced to

consumption of white radish sprouts. In 2005 an outbreak affecting 120 people on the west coast of Sweden was linked to iceberg lettuce and in 2006 a severe outbreak that affected many parts of the United States was traced to contamination of pre-packed spinach from California (reviewed in Holden *et al*., 2009; Little and Gillespie 2008).

Numerous studies have investigated the potential sources of vegetable contamination both pre-harvest in the field and post-harvest in the food chain. During the pre-harvest phase, the potential exists for persistent pathogen populations to establish on growing crops and then the risk to be amplified postharvest either by further direct contamination or by proliferation of existing pathogen populations during processing and handling procedures. Contamination in the field may occur because of application of inadequately composted manures or sewage, insect transmission, water run-off from nearby animal pastures or exposure to contaminated feces of wild animals.

The risk associated with using water from a range of sources and microbiological qualities for irrigation of salads and vegetables has been assessed and the need for improved guidelines recognized (Hamilton *et al*., 2006). Certainly in lettuce plants, surface irrigation and spray irrigation with suspensions of. *E. coli* O157:H7 lead to recovery of the pathogen from lettuce tissue 20 days later. The lettuce remained contaminated with *E. coli* O157:H7 even after washing, indicating that irrigation of food crops with water of unknown microbiological quality poses significant hazards. In the UK and Europe, legislation has moved responsibility for fresh produce safety away from government and into the supply chain, and retailers and producers have made significant progress in driving safety in production procedures through use of their own or other recognized standards (Monaghan, 2006). However, despite these efforts significant food poisoning incidents related to consumption of fresh produce

are still reported, indicating that under certain conditions inoculation and survival of human pathogens continues to occur.

There have been a very large number of studies of the persistence of human pathogens on plants. Most studies have focused on the survival of *E. coli* (largely *E. coli* O157:H7) and *Salmonella* sprayed or applied directly on to foliage of plants in some way, or applied on seeds, roots or into soil. With so many different experimental systems it is difficult to make general statements concerning population dynamics and survival of human pathogens on crop plants. Nevertheless, when applied directly to foliage or into soil, both *E. coli* and *Salmonella* are able to survive for extensive periods of time: >100 days on foliage and >5 months in soil (reviewed in Holden *et al.,* 2009). Importantly, standard post-harvest decontamination procedures with solutions containing approximately 20-200 μg/ml free chlorine for various lengths of time can reduce bacterial numbers but do not eliminate either the natural microbial population or human pathogens completely.

The phyllosphere is characterized by a number of extreme and often fluctuating environmental conditions combined with unique physio-chemical characteristics, to which typical phyllosphere microorganisms show a number of adaptations, allowing them to grow in these habitats. Human pathogens are not normally considered a part of the phyllosphere microbial population but it is clear that they do occur, as evidenced by the outbreaks of food poisoning described above. We recently reviewed the ecology of human pathogens in relation to the phyllosphere (Whipps *et al.,* 2008) and concluded that human pathogens can survive successfully in the phyllosphere of crop plants. We identified a need to understand the bacterial factors controlling attachment to intact and cut plant surfaces, the role of genetic variation in plants for a propensity for colonization of the phyllosphere by human pathogens and to identify

those plant characteristics which could be manipulated through crop genetics to decrease pathogenic bacteria attachment and survival.

While environmental and genetic factors play a critical role in determining patterns of phyllosphere colonization by bacteria, including human pathogens, much less is known of plant related factors which determine the potential for human pathogens to colonize, grow or survive as epiphytes or endophytes on or in leaves. Plant defense mechanisms have been shown to respond to the presence of such pathogens. Genome-wide analysis of the transcriptional response of *A. thaliana* to a plant pathogen and *E. coli* O157:H7 infiltrated into the leaf tissues identified many genes in common, suggesting the presence of common effector molecules in both bacteria (Thilmony *et al.*, 2006). Development of the microbial population in the phyllosphere will also be determined by phenotypic characteristics of the plant, certainly, there are "hot-spots" of microbial growth on the leaf associated with specific sites. Gross plant morphology is known to influence the size of phyllosphere bacterial populations and variation in bacterial populations between different species have been attributed to a range of plant characteristics, including leaf water content, leaf P content, amounts of bacteria-inhibitory phenolics, and leaf and mesophyll thickness (Yadav *et al.,* 2005). There have been very few studies of plant cultivar effects on human pathogens: however a recent report demonstrated a differential interaction between lettuce cultivars and *Salmonella enterica* serovars (Klerks *et al.,* 2007).

However, such a complex interaction is likely to be under the control of multiple plant and bacterial genes. An understanding of the mechanisms involved could lead to a reduction in pathogenic microorganisms on fresh produce and thereby a decrease in the risk of food-borne illness. A powerful approach to understanding and exploiting the underlying plant genetics would be quantitative genetic analysis on selected plant

species using appropriate experimental mapping populations. Genetic analysis to identify quantitative trait loci (QTL) allows the dissection of continuous phenotypic variation into contributions from discrete genetic factors based on the use of DNA markers. To do this a suitable mapping population is required with a clear differential parental response to the trait(s) of interest for construction of a genetic linkage map and also for the collection of 'robust' phenotypic data for the traits through properly designed replicated experiments. It is possible to determine whether QTL are specific to certain plant tissues, developmental stages or structures. For example, a study on shelf life of 'baby leaf' lettuce identified QTL for properties such as cell wall strength, stomatal index and epidermal cell area and suggested that bacterial colonization could play a role in post-harvest performance. As discussed earlier, plant characteristics such as these could indeed have a significant effect on pathogenic bacteria.

In conclusion, in face of the continuing potential risk to human health from consumption of contaminated fresh produce, there is a need to investigate microbe – plant interactions at both the phenotypic, genetic and molecular levels. Performing parallel complementary studies on the pathogen and the host crop will allow a better understanding of the factors affecting colonization of the phyllosphere by human pathogens which will ultimately form the basis of development of better control and decontamination strategies leading to production of safer produce.

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