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Fresh produce as a potential vector for bacterial human pathogens

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4 The predicted massive growth in the world overall population and the proportion of
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6 the elderly together with climate change and water shortages pose major global
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8 challenges in ensuring food security, both in terms of availability and safety. The
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10 geographic and demographic changes are likely to force unprecedented changes in
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12 land usage and agricultural practices (i.e. bringing closer together animal and crop
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14 production and the need to recycle water), which might increase the risk of
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16 contamination. Fresh produce, particularly salad leaves that are consumed raw, is
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18 becoming an increasingly important source of human infections. Despite the
19
20 increasing risk to public health little is currently known about the mechanism though
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22 which human pathogens bind to leaf surfaces or the plant traits that facilitate bacterial
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24 attachment.
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30 Enteric pathogens such as enterohaemorrhagic *E. coli* (EHEC) O157:H7 and
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32 *Salmonella enterica*, which are ranked as key food-borne pathogens of humans, are
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34 often attributed to consumption or handling of contaminated bovine or chicken
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36 products. However, recent outbreaks of food poisoning have been associated with
37
38 consumption of contaminated vegetable or salad produce and evidence suggests that
39
40 such outbreaks are increasing (reviewed in Holden *et al.*, 2009; Little and Gillespie
41
42 2008). Examples of international outbreaks of *S. enterica* and EHEC O157 linked to
43
44 ready-to-eat plant produce include a Scandinavian/UK outbreak of *S. Thompson*
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46 infection associated with consumption of rocket leaves; a Danish outbreak of *S.*
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48 *Anatum* infection linked to imported basil an outbreak of *S. Typhimurium* DT204b in
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50 several European countries associated with consumption of lettuce and an outbreak of
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52 *S. Senftenberg* infection associated with imported Israeli basil affecting the UK,
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54 Denmark, the Netherlands and the USA (Pezzoli *et al.*, 2008). The largest EHEC
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56 O157 outbreak to date, in 1996 in Sakai City, Osaka, Japan, was traced to
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3 consumption of white radish sprouts. In 2005 an outbreak affecting 120 people on the
4 west coast of Sweden was linked to iceberg lettuce and in 2006 a severe outbreak that
5 affected many parts of the United States was traced to contamination of pre-packed
6 spinach from California (reviewed in Holden *et al.*, 2009; Little and Gillespie 2008).
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10 Numerous studies have investigated the potential sources of vegetable contamination
11 both pre-harvest in the field and post-harvest in the food chain. During the pre-harvest
12 phase, the potential exists for persistent pathogen populations to establish on growing
13 crops and then the risk to be amplified postharvest either by further direct
14 contamination or by proliferation of existing pathogen populations during processing
15 and handling procedures. Contamination in the field may occur because of application
16 of inadequately composted manures or sewage, insect transmission, water run-off
17 from nearby animal pastures or exposure to contaminated feces of wild animals.
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22 The risk associated with using water from a range of sources and microbiological
23 qualities for irrigation of salads and vegetables has been assessed and the need for
24 improved guidelines recognized (Hamilton *et al.*, 2006). Certainly in lettuce plants,
25 surface irrigation and spray irrigation with suspensions of *E. coli* O157:H7 lead to
26 recovery of the pathogen from lettuce tissue 20 days later. The lettuce remained
27 contaminated with *E. coli* O157:H7 even after washing, indicating that irrigation of
28 food crops with water of unknown microbiological quality poses significant hazards.
29
30 In the UK and Europe, legislation has moved responsibility for fresh produce safety
31 away from government and into the supply chain, and retailers and producers have
32 made significant progress in driving safety in production procedures through use of
33 their own or other recognized standards (Monaghan, 2006). However, despite these
34 efforts significant food poisoning incidents related to consumption of fresh produce
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3 are still reported, indicating that under certain conditions inoculation and survival of
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5 human pathogens continues to occur.
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9 There have been a very large number of studies of the persistence of human
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11 pathogens on plants. Most studies have focused on the survival of *E. coli* (largely *E.*
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13 *coli* O157:H7) and *Salmonella* sprayed or applied directly on to foliage of plants in
14
15 some way, or applied on seeds, roots or into soil. With so many different experimental
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17 systems it is difficult to make general statements concerning population dynamics and
18
19 survival of human pathogens on crop plants. Nevertheless, when applied directly to
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21 foliage or into soil, both *E. coli* and *Salmonella* are able to survive for extensive
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23 periods of time: >100 days on foliage and >5 months in soil (reviewed in Holden *et*
24
25 *al.*, 2009). Importantly, standard post-harvest decontamination procedures with
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27 solutions containing approximately 20-200 µg/ml free chlorine for various lengths of
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29 time can reduce bacterial numbers but do not eliminate either the natural microbial
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31 population or human pathogens completely.
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37 The phyllosphere is characterized by a number of extreme and often fluctuating
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39 environmental conditions combined with unique physio-chemical characteristics, to
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41 which typical phyllosphere microorganisms show a number of adaptations, allowing
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43 them to grow in these habitats. Human pathogens are not normally considered a part
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45 of the phyllosphere microbial population but it is clear that they do occur, as
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47 evidenced by the outbreaks of food poisoning described above. We recently reviewed
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49 the ecology of human pathogens in relation to the phyllosphere (Whipps *et al.*, 2008)
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51 and concluded that human pathogens can survive successfully in the phyllosphere of
52
53 crop plants. We identified a need to understand the bacterial factors controlling
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55 attachment to intact and cut plant surfaces, the role of genetic variation in plants for a
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57 propensity for colonization of the phyllosphere by human pathogens and to identify
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3 those plant characteristics which could be manipulated through crop genetics to
4 decrease pathogenic bacteria attachment and survival.
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8 While environmental and genetic factors play a critical role in determining patterns of
9 phyllosphere colonization by bacteria, including human pathogens, much less is
10 known of plant related factors which determine the potential for human pathogens to
11 colonize, grow or survive as epiphytes or endophytes on or in leaves. Plant defense
12 mechanisms have been shown to respond to the presence of such pathogens. Genome-
13 wide analysis of the transcriptional response of *A. thaliana* to a plant pathogen and *E.*
14 *coli* O157:H7 infiltrated into the leaf tissues identified many genes in common,
15 suggesting the presence of common effector molecules in both bacteria (Thilmony *et al.*,
16 2006). Development of the microbial population in the phyllosphere will also be
17 determined by phenotypic characteristics of the plant, certainly, there are “hot-spots”
18 of microbial growth on the leaf associated with specific sites. Gross plant morphology
19 is known to influence the size of phyllosphere bacterial populations and variation in
20 bacterial populations between different species have been attributed to a range of
21 plant characteristics, including leaf water content, leaf P content, amounts of bacteria-
22 inhibitory phenolics, and leaf and mesophyll thickness (Yadav *et al.*, 2005). There
23 have been very few studies of plant cultivar effects on human pathogens: however a
24 recent report demonstrated a differential interaction between lettuce cultivars and
25 *Salmonella enterica* serovars (Klerks *et al.*, 2007).
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51 However, such a complex interaction is likely to be under the control of multiple plant
52 and bacterial genes. An understanding of the mechanisms involved could lead to a
53 reduction in pathogenic microorganisms on fresh produce and thereby a decrease in
54 the risk of food-borne illness. A powerful approach to understanding and exploiting
55 the underlying plant genetics would be quantitative genetic analysis on selected plant
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3 species using appropriate experimental mapping populations. Genetic analysis to
4 identify quantitative trait loci (QTL) allows the dissection of continuous phenotypic
5 variation into contributions from discrete genetic factors based on the use of DNA
6 markers. To do this a suitable mapping population is required with a clear differential
7 parental response to the trait(s) of interest for construction of a genetic linkage map
8 and also for the collection of 'robust' phenotypic data for the traits through properly
9 designed replicated experiments. It is possible to determine whether QTL are specific
10 to certain plant tissues, developmental stages or structures. For example, a study on
11 shelf life of 'baby leaf' lettuce identified QTL for properties such as cell wall
12 strength, stomatal index and epidermal cell area and suggested that bacterial
13 colonization could play a role in post-harvest performance. As discussed earlier, plant
14 characteristics such as these could indeed have a significant effect on pathogenic
15 bacteria.
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34 In conclusion, in face of the continuing potential risk to human health from
35 consumption of contaminated fresh produce, there is a need to investigate microbe –
36 plant interactions at both the phenotypic, genetic and molecular levels. Performing
37 parallel complementary studies on the pathogen and the host crop will allow a better
38 understanding of the factors affecting colonization of the phyllosphere by human
39 pathogens which will ultimately form the basis of development of better control and
40 decontamination strategies leading to production of safer produce.
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