REVIEW ARTICLE

Colonization outwith the colon: plants as an alternative environmental reservoir for human pathogenic enterobacteria

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Received 21 August 2008; revised 17 October 2008; accepted 26 October 2008.
Final version published online 9 December 2008.

DOI:10.1111/j.1574-6976.2008.00153.x

Editor: Eva Top

Keywords
human pathogenic enterobacteria; fresh produce; plant hosts; plant colonization.

Abstract

Members of the *Enterobacteriaceae* have the capacity to adapt to a wide variety of environments and can be isolated from a range of host species across biological kingdoms. Bacteria that are pathogenic to animals, in particular humans, are increasingly found to be transmitted through the food chain by fruits and vegetables. Rather than simply contaminating plant surfaces, there is a growing body of evidence to show that these bacteria actively interact with plants and can colonize them as alternative hosts. This review draws together evidence from studies that investigate proven and potential mechanisms involved in colonization of plants by human pathogenic enterobacteria.

Introduction

The *Enterobacteriaceae* are a family of mesophilic bacteria that are found in a diverse variety of environments, terrestrial and aquatic, and in a broad range of host species, both plant and animal. Interactions with host species may be benign, mutualistic or pathogenic, and the family contains many important pathogens of both animals and plants. For example, salmonellae and verotoxigenic *Escherichia coli* (VTEC) (together with non-enterobacterial *Campylobacter* spp.) are among the most prevalent food-borne bacterial pathogens in the developed world, and are able to enter the food chain at any point (Fisher & Threlfall, 2005). The main animal reservoirs include cattle for VTEC (Elder *et al.*, 2000), poultry for *Salmonella enterica* serovar Enteritidis (*S.* Enteritidis) (de Buck *et al.*, 2004) and pigs for *S. enterica* serovar Typhimurium (*S.* Typhimurium) (Davies *et al.*, 2004). These associations are commonly understood, but less well known is that animal pathogenic enterobacteria are also able to colonize a much wider range of hosts, including plants. This is perhaps unsurprising, as plants are the primary hosts for many other enterobacteria, which behave as epiphytes, endophytes and/or pathogens. These include the genera *Erwinia*, *Pectobacterium*, *Dickeya*, *Pantoea*, *Enterobacter* and *Brenneria* (Hauben *et al.*, 1998; Samson *et al.*, 2005). Recent studies on the enterobacterial plant pathogen *Pectobacterium atrosepticum* and the plant-associated enterobacterium *Klebsiella pneumoniae* have shown that a remarkably high proportion of their genome is shared with the human pathogenic enterobacteria (Bell *et al.*, 2004; Toth *et al.*, 2006; Fouts *et al.*, 2008; and this study). In fact, cross-kingdom jumps have been recorded for many members of the *Enterobacteriaceae*. For example, *E. coli* O157:H7 proliferates quite extensively in the mouth parts of the common house fly (Kobayashi *et al.*, 1999); the plant pathogens *Dickeya dadantii* and *Pantoea ananatis* also cause diseases in pea aphids and humans, respectively (Grenier *et al.*, 2006), while the endophyte/plant pathogen *Pantoea agglomerans* and endophyte *K. pneumoniae* have both been associated with opportunistic infections in animals, including humans (Garcia de la Torre *et al.*, 1985; Dong *et al.*, 2001; de Baere *et al.*, 2004; Cruz *et al.*, 2007; Morales-Valenzuela *et al.*, 2007; Mano & Morisaki, 2008). Such cross-kingdom jumps have been considered for a wide range of pathogens in more detail in van Baarlen *et al.* (2007).

Incidence of food-borne infections associated with fresh produce

The ability of animal pathogenic enterobacteria to contaminate nonmeat products is an accepted fact. Foodstuffs as

diverse as unpasteurized fruit juice, dairy products, dried herbs and spices all act as vehicles for transmission. Fresh fruit and vegetables are now recognized to be a major route of entry for animal pathogenic enterobacteria into the food chain. One of the largest outbreaks of VTEC-derived gastroenteritis occurred in Japan in 1996 as a result of contamination of radish sprouts with *E. coli* O157:H7 (Michino *et al.*, 1999; Watanabe *et al.*, 1999). A recent study found that almost one quarter of all reported cases of VTEC in the United States were associated with fruits and vegetables (Rangel *et al.*, 2005). Several large-scale outbreaks have occurred recently. In 2005/2006, more than 450 cases of salmonellosis, in four separate outbreaks, were reported in Canada and the United States arising from contaminated tomatoes (Bidol *et al.*, 2007). A similar situation has occurred again in 2008, with over 1300 reported cases in an outbreak of salmonellosis associated with tomatoes and peppers (ongoing at the time of this review: http://www.cdc.gov/salmonella/saintpaul/). In the United States in 2006 an outbreak of *E. coli* O157:H7 across several US states occurred as a result of the contamination of fresh spinach, with more than 200 reported cases of infection and three fatalities (Anon, 2006). In the latter half of 2007, *S. enterica* serovar Paratyphi associated with baby spinach and leafy vegetable salad infected at least 430 individuals in northern Europe (Denny *et al.*, 2007). Changes in farming practices, food production, consumer habits and greater vigilance in surveillance are all possible explanatory factors in the increase in incidence of animal pathogenic enterobacteria from fresh produce. However, the significance of plants as alternative hosts and, as such, additional reservoirs for pathogenic bacteria, is becoming clearer.

Prevalence of animal-associated enterobacteria on plants

The current paradigm is that any association of animal pathogenic enterobacteria with fresh produce is most likely a result of cross-contamination from animals or meat products. Undoubtedly, cross-contamination is an important factor, both on the farm and during food processing. Animal slurry or manure may carry pathogenic bacteria directly into soil or watercourses; alternatively contaminated meat products may cross-contaminate fresh produce during food preparation. However, plant material does not simply represent an inert surface on which to transmit pathogenic bacteria, but rather constitutes a complex and responsive biological system. Not surprisingly, therefore, an increasing number of recent studies have demonstrated that this interaction is dynamic and specific, and effectively identifies some plant species as alternative hosts for the animal pathogenic enterobacteria.

Reports from laboratory- and field-based studies have yet to show a definitive niche for animal pathogenic enterobacteria either within or on plants, although it does appear that the bacteria preferentially colonize plant roots and the surrounding rhizosphere (described below). This is presumably because bacteria are protected from desiccation and harmful UV radiation on and around roots, but, perhaps more importantly, because this location provides access to a nutrient-rich environment (Bais *et al.*, 2006). Water availability, an important factor for bacterial motility along surfaces, is also greater in the rhizosphere than on foliage, although bacteria are still capable of migrating along the aerial surfaces of plants. Human pathogenic enterobacteria that were inoculated into the compost of soil-grown *Arabidopsis thaliana* were shown to migrate up the stem along the surface of the plants, and were subsequently isolated from leaves and flowers (Cooley *et al.*, 2003). These bacteria also internalized within plant tissue, mainly in the roots, where they were then able to migrate within the plant tissue to the foliage. The ability to internalize within plant tissue is not reported in laboratory studies alone, as human pathogenic enterobacteria have been isolated from within the tissue of fresh and minimally prepared produce (Eblen *et al.*, 2004; Shi *et al.*, 2007; Soto *et al.*, 2007). Furthermore, it appears that once a plant has been colonized there is the potential for vertical transmission to successive generations, as demonstrated for *S.* Typhimurium on tomatoes (Guo *et al.*, 2001). Although there is insufficient evidence in the literature to demonstrate host species specificity, it has been possible to detect human pathogenic enterobacteria on a very wide range of plant tissues, from fruiting bodies to cereal grains. For a comprehensive list of fresh produce that has been linked to a variety of infectious agents see (Beuchat, 1996).

Evidence that human pathogenic enterobacteria can colonize plants preharvest has been shown using artificially contaminated manure or irrigation water, either in environmentally controlled growth chambers or experimental field plots. Lettuce plants that were grown in manure amended with fluorescently marked *E. coli* O157:H7 were shown to harbour bacteria that had internalized into the plant tissue. Lettuce foliage was either surface sterilized before internalized bacteria were enumerated from the plants on agar plates, or the bacteria were visualized within leaf tissue by confocal microscopy (Solomon *et al.*, 2002). Internalization of *E. coli* O157:H7 occurred when the plants were grown in soil containing contaminated manure or animal slurry or were irrigated with contaminated water. Long-term persistence of *E. coli* O157:H7 in fresh produce has been demonstrated with carrots and onions grown in artificially contaminated manure compost (Islam *et al.*, 2004). This study showed that the bacteria could be detected from carrots for up to 12 weeks after initial application and in onions up to 9 weeks. A similar study showed that

S. enterica could be detected in tomato plants 7 weeks after the seeds were sown in soil artificially contaminated with the bacteria (Barak & Liang, 2008).

In general, similar strategies are required for bacteria to colonize any host, whether plant or animal. The processes of initial adherence, invasion and establishment may differ in terms of the specific interactions, but many parallels and similarities exist. A combination of bioinformatic approaches and molecular techniques have been used to study mechanisms of plant colonization by pathogenic enterobacteria. Pathogenicity and the colonization capacity of enterobacterial phytopathogens such as *P. atrosepticum* have been explored in the context of their relationship with other members of the *Enterobacteriaceae* (Toth *et al.*, 2006), while other researchers have provided discussions and reviews on the extent of plant colonization by human pathogenic bacteria (Beuchat, 1996; Berg *et al.*, 2005; Brandl, 2006). The focus of this review is the molecular basis of the interactions at different stages of colonization by human pathogenic enterobacteria. We consider laboratory evidence that indicates direct interactions and speculate on other factors that may also be relevant to colonization of both plant and animal tissues.

Genomic comparisons

Description of genomic comparisons

The extensive availability of sequenced bacterial genomes permits *in silico* genomic comparisons between bacteria that are frequently or exclusively associated with plants and those that are associated with human or animal hosts. In particular, it is possible to extend BLAST and other sequence alignment-based searches beyond the identification of homologues for single genes to large-scale comparisons of whole bacterial genomes. A number of comparative techniques are appropriate for such comparisons, including the identification of putative orthologous genes by reciprocal best hit (RBH) analyses. An RBH is defined as a pair of sequences, one from each genome, which are each the best match for the other as determined by the sequence alignment method used (Moreno-Hagelsieb & Latimer, 2008). RBHs in a query and comparator organism are normally interpreted to indicate putative orthologues. The alignments and results from RBH analyses can be visualized using programs such as GENOMEDIAGRAM (Pritchard *et al.*, 2006) (Fig. 1).

Where two organisms contain putative orthologues, i.e. RBH, with sufficient sequence identity, it is to be expected that those orthologues are likely to perform similar biochemical functions. While it is not necessarily the case that two organisms able to perform a particular biochemical function share orthologous genes that encode that function, this is likely for organisms that share a common ancestor. In particular, phylogenetically closely related organisms are expected to share sets of RBH that encode functions that are shared with a common ancestor. Where functional genes have been acquired from an organism that does not share a recent common ancestor with the reference sequence, it would be unlikely to find RBH for those genes in phylogenetically related organisms. However, such genes may be present in organisms that share a common environmental niche with the reference organism. From this it is possible to infer that genes in a reference organism that are putatively orthologous to similar genes in closely related organisms are likely to be inherited from a common ancestor. On the other hand, those that are instead orthologous (or otherwise more similar) to genes in less closely related organisms might indicate lateral gene transfer. Where such organisms also share an environmental niche with the reference bacterium, for example the plant environment, it can be postulated that those genes code for functions that are advantageous in that niche. Figure 1 indicates such comparisons for *K. pneumoniae* and several animal and plant pathogenic bacteria (explained in more detail below).

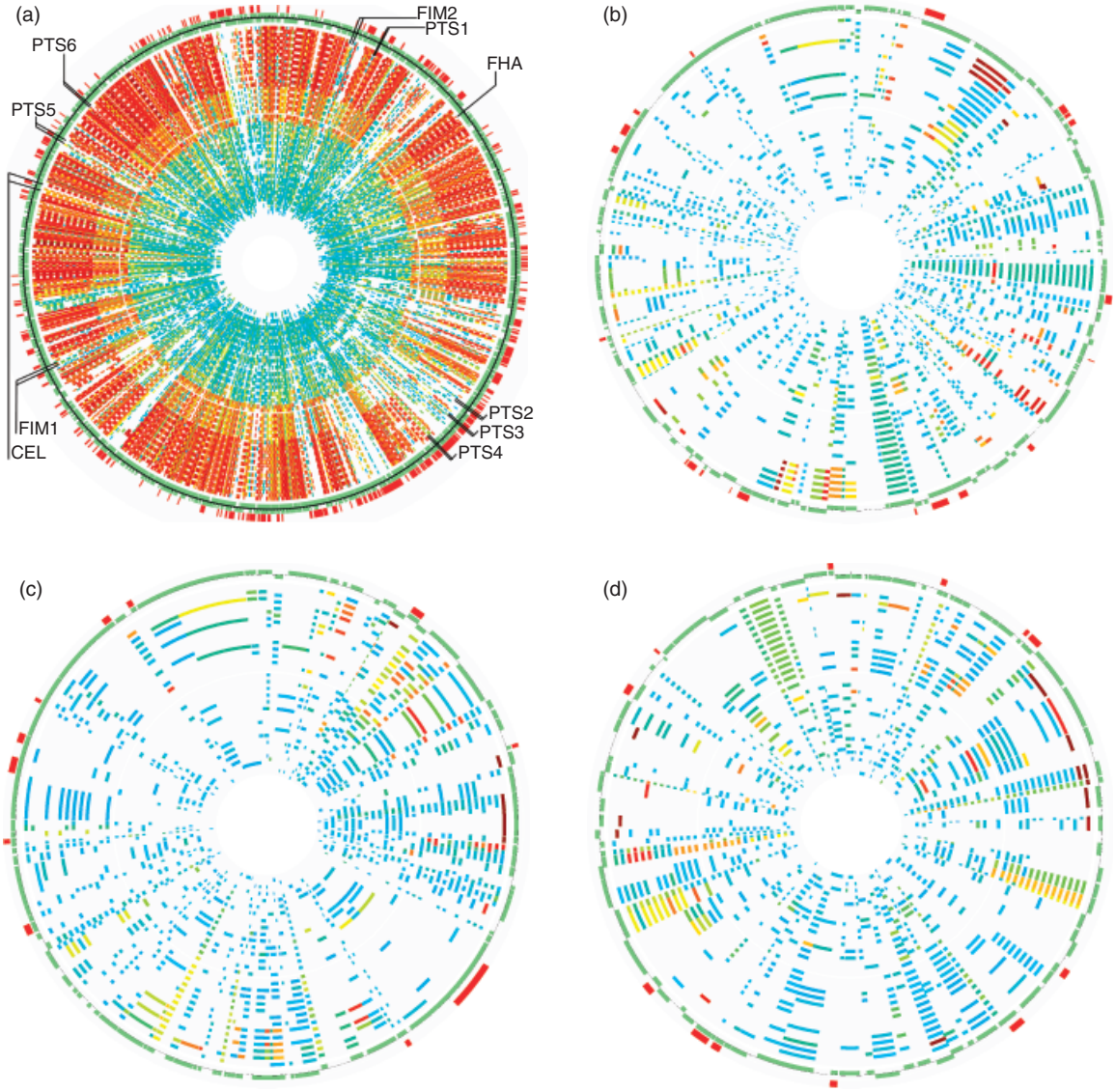
Value in exploring host–microorganism interactions

RBH analysis applied to the plant pathogen *P. atrosepticum* indicated the presence of a large number of coding sequences (CDS) that have putative orthologues in plant-associated bacteria, but not in other enterobacteria (Bell *et al.*, 2004; Toth *et al.*, 2006). *Pectobacterium atrosepticum* shares a high-average protein sequence identity to animal-associated enterobacteria, which recapitulates the phylogenetic history of *P. atrosepticum* within the *Enterobacteriaceae*. However, $>$ 10% of the *P. atrosepticum* CDS have RBH in plant-associated bacteria, which are either not present in animal-associated enterobacteria or have RBH in animal-associated species that are significantly less similar to the query sequence than their counterparts in plant-associated bacteria. This may imply a role for these CDS in plant–bacteria interactions (Toth *et al.*, 2006), and recent work has shown this to be the case for some of these CDS (unpublished data). Similar analyses have been carried out for enterobacteria thought to be limited to animal hosts. Comparisons with the genome of *S. enterica* serovar Typhi, for example, indicated that only a small number of chromosomal CDS have RBH in plant-associated bacteria (Toth *et al.*, 2006). This may reflect a requirement of niche adaptation for *S.* Typhi, which appears to have specialized or is in the process of specialization towards human hosts. The genome is characterized by gene reduction and the presence of large numbers of pseudogenes, in contrast to the genomes of more generalist enterobacteria (Parkhill *et al.*, 2001). However, a dispropor-

tionately large number of sequences on one of the two plasmids carried by *S.* Typhi have RBH in plant-associated bacteria, but not in other enterobacteria (Toth *et al.*, 2006). The plasmid is mobile and can transfer between *S.* Typhi strains and into *E. coli* (under laboratory conditions) (Wain *et al.*, 2003). It also contains several mobile genetic elements such as insertion elements. Thus, it is possible that some genes may have been transferred indirectly from plant-associated bacteria through a series of horizontal transfer events.

Unlike specialized pathogens, such as *S.* Typhi, genomic comparisons carried out using the sequences of more generalist animal pathogenic enterobacteria have a greater proportion of RBH that are common with plant-associated bacteria. Therefore, it is possible that some of these putatively orthologous genes contribute to colonization of plant hosts. For example, the *E. coli* O157:H7 Sakai strain was originally isolated from a large-scale outbreak associated with radish sprouts (Watanabe *et al.*, 1999). A comparison with plant-associated bacteria reveals that a relatively large number of RBHs are shared with plant-associated bacteria, but not with animal-associated enterobacteria. These RBHs are often clustered together on the chromosome, indicative of horizontal gene transfer (unpublished data). Analysis of *S.* Typhimurium (strain LT2) reveals a similarly large proportion of RBHs, again often clustered in discrete islands.

Klebsiella pneumoniae is a remarkable member of the family, in that it is seen clearly to cross the plant/animal boundary, where it readily colonizes plant hosts, but is



equally capable of exploiting human hosts under certain conditions (Garcia de la Torre *et al.*, 1985; Dong *et al.*, 2001). For example, certain *Klebsiella* strains have genes encoding the components of nitrogen fixation, a process that underpins their plant-associated lifestyle. Many *Klebsiella* strains are also the causative agents of hospital-acquired and urinary tract infections in humans. This dual-host behaviour requires considerable genetic flexibility and plasticity, as discussed recently (Fouts *et al.*, 2008). An RBH comparison with plant-associated bacteria showed a high level of RBH to plant-associated, but not animal-associated bacteria. Images from the analyses of the *K. pneumoniae* chromosome and three plasmids using GENOMEDIAGRAM have been presented to give a visual comparison of the number and location of the RBHs (Fig. 1).

The GENOMEDIAGRAM highlights four categories of genes in *K. pneumoniae* (strain MGH78578): (1) well-conserved genes that are present across both sets of comparator genomes; (2) genes in the query genome that are unique and do not show RBH to any comparator; (3) genes that are shared with just animal-associated bacteria and (4) genes that are shared with just plant-associated bacteria. Detecting group (4) is the aim of this analysis and their locations have been indicated by a red mark on the circular genome of *K. pneumoniae* on Fig. 1. While a large proportion of RBHs encode hypothetical proteins or are phage derived, it is still possible to make biological inferences from the analysis with genes that have known or putative functions. RBHs that are shared between plant-associated bacteria and *K. pneumoniae*, but are not present in other animal-associated enterobacteria, include several carbohydrate uptake and catabolism genes that are likely to facilitate the use of carbohydrates directly from plants. For example, among the RBHs are six (putative) phosphotransferase systems (PTS) that are required for catabolism of carbohydrates characteristic of plant material, including cellobiose, fructose and galactose (Fig. 1a labelled PTS 1–6) (Ward & Moo-Young, 1989). To contrast, two fimbrial clusters that are shared with animal-associated enterobacteria, but are not present in plant-associated bacteria have also been indicated on Fig. 1a (FIM1 and FIM2). Both sets of genes are located in regions that are not shared by the complete set of comparators and are, therefore, most likely indicative of horizontal transfer. Other genes that are discussed later in the text and are likely to be involved in adherence have also been indicated on Fig. 1: two clusters for synthesis of

Fig. 1. Circular representations of the *Klebsiella pneumoniae* chromosome strain MHG78578 (a) and plasmids pKPN3 (b), pKPN4 (c) and pKNP5 (d), indicating CDS, RBHs for each CDS to other bacterial genomes, and the ratio of percent amino acid sequence identity in plant-associated bacteria to animal-associated bacteria for each CDS. PTS of the *K. pneumoniae* strain MGH78578 have been labelled PTS 1–6, fimbrial features labelled FIM 1 and 2, cellulose synthesis genes labelled CEL and an agglutinin labelled FHA. The inner rings indicating RBHs to CDSs are coloured individually, on a scale from 30% (cyan) to 100% amino acid identity (brick red). From the outer edge, successive rings represent: CDSs (marked in red) for which the P : E ratio (mean percentage identity to RBHs in plant-associated bacteria : mean percentage identity of each CDS to RBHs in animal-associated bacteria) ≥ 1.5; CDSs from the reference sequence in forward and reverse directions, coloured in green; RBHs to CDSs from other bacterial chromosomes, in order of decreasing mean percentage identity per CDS. The comparator genomes are arranged so that animal-associated bacteria are closest to the outer ring and plant-associated bacteria are closest to the middle. A clear line divides both groups. The order of the comparator genomes is as follows: For (a): *Shigella flexneri* 2a (2457T), *Salmonella enterica* serovar Paratyphi A [American Type Culture Collection (ATCC) 9150), *S. enterica* serovar Typhimurium (LT2), *S. enterica* serovar Typhi (Ty2), *S. flexneri* 2a (301), *Escherichia coli* O157:H7 (EDL933), *E. coli* O157:H7 (Sakai), *E. coli* (CFT073), *S. enterica* serovar Typhi (CT18), *Yersinia pestis* (KIM), *Y. pestis* (CO92), *Y. pestis* biovar Medievalis (91001), *Yersinia pseudotuberculosis* (IP 32953), *Pectobacterium atrosepticum* (SCRI1043), *Pseudomonas putida* (KT2440), *Pseudomonas syringae* pv. *tomato* (DC3000), *Xanthomonas campestris* pv. *campestris* (ATCC 33913), *Xanthomonas axonopodis* pv. *citri* (306), *Xanthomonas oryzae* pv. *oryzae* (KACC10331), *Xylella fastidiosa* (Temecula1), *Ralstonia solanacearum* (GMI1000), *X. fastidiosa* (9a5c), *Bradyrhizobium japonicum* (USDA 110), *Mesorhizobium loti* (MAFF303099), *Agrobacterium tumefaciens* (C58), *Sinorhizobium meliloti* (1021), *Leifsonia xyli* ssp. *xyli* (CTCB07), Onion yellows phytoplasma (OY-M 15). For (b) *E. coli* O157:H7 (Sakai), *E. coli* O157:H7 (EDL933), *E. coli* (CFT073), *S. flexneri* 2a (301), *S. flexneri* 2a (2457T), *S.* Typhi (CT18), *S.* Typhimurium (LT2), *S.* Typhi (Ty2), *Y. pestis* biovar Medievalis (91001), *S.* Paratyphi A (ATCC 9150), *Y. pestis* (CO92), *Y. pseudotuberculosis* (IP 32953), *Y. pestis* (KIM), *P. atrosepticum* (SCRI1043), *P. putida* (KT2440), *P. syringae* pv. *tomato* (DC3000), *R. solanacearum* (GMI1000), *B. japonicum* (USDA 110), *A. tumefaciens* (C58), *M. loti* (MAFF303099), *S. meliloti* (1021), *X. oryzae* pv. *oryzae* (KACC10331), *X. campestris* pv. *campestris* (ATCC 33913), *X. fastidiosa* (9a5c), *X. axonopodis* pv. *citri* (306), *X. fastidiosa* (Temecula1), *L. xyli* ssp. *xyli* (CTCB07), Onion yellows phytoplasma (OY-M 15). For (c) *S.* Typhi (CT18), *E. coli* O157:H7 (Sakai), *S. flexneri* 2a (301), *Y. pestis* (CO92), *E. coli* (CFT073), *Y. pestis* biovar Medievalis (91001), *S.* Paratyphi A (ATCC 9150), *S.* Typhimurium (LT2), *Y. pestis* (KIM), *Y. pseudotuberculosis* (IP 32953), *S. flexneri* 2a (2457T), *E. coli* O157:H7 (EDL933), *S.* Typhi (Ty2), *P atrosepticum* (SCRI1043), *P. syringae* pv. *tomato* (DC3000), *B. japonicum* (USDA 110), *X. axonopodis* pv. *citri* (306), *S. meliloti* (1021), *X. fastidiosa* (9a5c), *X. fastidiosa* (Temecula1), *X. oryzae* pv. *oryzae* (KACC10331), *P. putida* (KT2440), *M. loti* (MAFF303099), *R. solanacearum* (GMI1000), *X. campestris* pv. *campestris* (ATCC 33913), *A. tumefaciens* (C58), *L. xyli* ssp. *xyli* (CTCB07), Onion yellows phytoplasma OY-M. For (d) *S. flexneri* 2a (2457T), *S. enterica* serovar Typhi (CT18), *E. coli* O157:H7 (Sakai), *S.* Typhimurium (LT2), *E. coli* O157:H7 (EDL933), *E. coli* (CFT073), *S.* Typhi Ty2, *S.* Paratyphi A (ATCC 9150), *S. flexneri* 2a (301), *Y. pestis* (CO92), *Y. pestis* biovar Medievalis (91001), *Y. pestis* (KIM), *Y. pseudotuberculosis* (IP 32953), *P. atrosepticum* (SCRI1043), *X. oryzae* pv. *oryzae* (KACC10331), *P. syringae* pv. *tomato* (DC3000), *X. axonopodis* pv. *citri* (306), *P. putida* (KT2440), *X. campestris* pv. *campestris* (ATCC 33913), *X. fastidiosa* (Temecula1), *S. meliloti* (1021), *A. tumefaciens* (C58), *B. japonicum* (USDA 110), *X. fastidiosa* (9a5c), *M. loti* (MAFF303099), *R. solanacearum* (GMI1000), *L. xyli* ssp. *xyli* (CTCB07), Onion yellows phytoplasma (OY-M). (Plasmids that do not demonstrate RBH are not shown.)

cellulose (labelled CEL) and a gene encoding an agglutinin (labelled FHA).

Whether CDS that have RBH to genes in plant-associated bacteria actually play a role in plant colonization remains to be determined empirically and is the subject of ongoing research in this laboratory. Sequences that have been identified by this method fall mostly into one of the two classes: a high proportion of the sequences have no functional annotation in GenBank; a second major group are phage-related genes. Genomic comparisons thus support the hypothesis that bacteriophages are drivers of bacterial evolution by transferring both viral and bacterial DNA between species. The hypothesis that genomes of related bacterial species are essentially mosaic in structure and that their evolution is a dynamic process involving both gain and loss of genomic units, is also supported by this analysis (Welch *et al.*, 2002; Tyson *et al.*, 2004; Pallen & Wren, 2007). Comparative genomics is a valuable tool, which provides a screening method to determine factors shared between plant- and animal-associated bacteria and, which has a possible role in bacteria–plant interactions.

Mechanisms of plant colonization

Adherence

Bacterial adherence to host tissue is a prerequisite of both animal and plant infection. Bacteria encode within their genomes a large number of adherence factors with diverse receptor-binding capability. Some of these define specificity for either host species or a particular host niche, whereas others exhibit lower affinity binding. Molecular Koch's postulates have been satisfied for adherence factors in animal model and plant infection systems that encompass a diversity of structures and export mechanisms. Examples include chaperone-usher assembled fimbrial adhesins, such as *E. coli* pyelonephritis associated pili (Bergsten *et al.*, 2005) and the haemagglutinin-like adhesin HecA encoded by *D. dadantii* (formerly *Erwinia chrysanthemi*) (Rojas *et al.*, 2002).

Adhesins of the chaperone-usher family are generally located on the ends of long hair-like structures termed fimbriae, or are surface associated, afimbrial adhesins. The adhesins recognize a range of glycosylation patterns that decorate host surface proteins. This class of fimbrial adhesins is relatively common among the enterobacteria, where different isolates commonly encode specific sets of adhesin gene clusters that, in turn, confer tropism to a particular host tissue type. We and others have found that the expression of adhesin gene clusters is differentially responsive to environmental cues, such as temperature. *Escherichia coli* O157:H7 encodes determinants for 16 different adhesin gene clusters, some of which play a role in animal host colonization and are optimally expressed at 37 °C. However, the expression of a distinct subset of adhesins was found to be upregulated at 28 °C, compared with 37 °C (Low *et al.*, 2006), which is consistent with a role in adherence to surfaces other than those found in animal hosts. Whether these adhesins play a role in colonization of alternative hosts, such as plants, is the focus of the ongoing research. Two fimbrial clusters of *K. pneumoniae* that are indicated on Fig. 1 are involved in adherence to animal tissue; type 1 fimbriae (Podschun *et al.*, 1993) and Stb fimbriae (homologous to *S. enterica stb* cluster) (Weening *et al.*, 2005). Perhaps unsurprisingly, plant pathogenic enterobacteria also encode fimbrial adhesin clusters, but their roles in plant colonization have yet to be characterized. Glycosylation is a common mechanism of post-translational modification of surface-expressed proteins in eukaryotes, and it is likely that bacteria will exploit similar mechanisms of adherence whether attaching to plant or animal cells (Wilson, 2002).

Fimbrial-like structures can also contribute to functions in pathogenesis other than adherence. A common mechanism used by pathogens to manipulate host defences is the injection of microbial effector proteins directly into host cells. In bacterial pathogens, effector proteins are secreted via the type III secretion system (TTSS), a needle-like complex that forms a junction between the pathogen and the host cell. In both plant and animal hosts, effector proteins are translocated through the complex into the host cell, where they may be involved in manipulating host defences. The TTSS shares common ancestry with the flagella export structural proteins and has evolved to be a very successful effector delivery mechanism in both plant pathogenic and animal pathogenic bacteria (Hueck, 1998). In pathogenic *E. coli*, the fimbrial-like structure of the TTSS firstly binds to host cells, mediated by the major structural protein EspA. Once bound to the host cell, a pore is formed in the host membrane and one of the first proteins to be translocated into the host cell is a receptor (Tir) that recognizes a bacterial adhesin (intimin). Binding of Tir to intimin results in a close association of bacterial and host cells and is the forerunner to the characteristic 'attaching and effacing' (A/E) lesion formation (Nougayrede *et al.*, 2003). EspA is required for A/E lesion formation *in vivo* and is involved in binding to bovine tissue (Naylor *et al.*, 2005; La Ragione *et al.*, 2006). A recent study suggests that *E. coli* EspA also facilitates binding to plant tissue (Shaw *et al.*, 2008). Two different pathotypes of *E. coli* were shown to adhere to the leaf epidermis of salad vegetables in an EspA-dependent manner. However, it should be noted that expression of the TTSS responds to multiple environmental cues and is temperature sensitive. Optimal expression of the TTSS in human pathogenic enterobacteria occurs at 37 °C, which corresponds to the temperature of animal reservoirs. Furthermore, the authors report that bacterial binding to

the leaf epidermis occurred at 37 °C, but not at 20 °C, a condition in which expression of the TTSS is downregulated (Shaw *et al*., 2008). Given the differences in host temperature, the significance of EspA in bacteria–plant interactions is yet to be determined. TTSS of phytopathogenic bacteria, which are fully adapted to plant hosts, display enhanced expression at lower temperatures corresponding to a plant-based lifestyle. For example, expression of the *Pseudomonas syringae* TTSS is enhanced at 20 °C relative to expression at 30 °C (Collmer *et al*., 2000).

In some bacteria, flagella share function as well as structure with the TTSS. In this group flagella provide a means of both locomotion and adherence. For example, flagella of enteropathogenic *E. coli* have been shown to enhance bacterial binding to human epithelial cells (Giron *et al*., 2002). The production of flagella appeared to increase in the presence of host cells and purified flagella bound directly to the cells (Giron *et al*., 2002). However, the interactions on human epithelial cells were specific and did not occur for H7 type, characteristic of O157 serotypes of *E. coli*. In other Gram-negative bacteria such as *Campylobacter jejuni*, flagella have also been shown to mediate adherence to animal hosts (Grant *et al*., 1993). It appears that flagella may also play a role in interactions with plant hosts. The ability of *Listeria monocytogenes* to bind to radish tubers was found to correlate with functional motility (Gorski *et al*., 2003). In addition, adhesion was temperature dependent and enhanced at temperatures < 37 °C, which correlates with *Listeria* flagellar expression. However, it was not possible to identify any known flagella genes in a screen of nonmotile mutants, which suggests an indirect effect in this case. Recent studies have shown that flagella mediate binding of *S. enterica* serovar Senftenberg to basil leaves at both 20 and 37 °C (Berger *et al*., 2008). Interestingly, the contribution of flagella to adherence was reduced for *S*. Typhimurium at 20 °C and flagella did not appear to play any role in binding at 37 °C. Further studies are required to determine the extent to which flagell'a of human pathogenic enterobacteria facilitate adherence to plant tissue.

Bacterial adherence to biotic (and abiotic) surfaces is normally due to a combination of adherence factors, rather than the action of a single one. Furthermore, bacteria are often found in complex communities, such as biofilms, which are dependent on a number of adherence factors (Van Houdt & Michiels, 2005). The biofilm matrix is made up of components that include polysaccharides, fimbriae, nucleic acids and agglutinins. Agglutinins are a family of very large surface-exposed proteins, shared by a diverse range of both Gram-positive and Gram-negative bacteria. Archetypal haemagglutinins are well characterized and in general, mediate the typical interactions with erythrocytes that provide their designation. They have also been reported to mediate other cell–cell interactions. For example, SiiE and BapA are two related *S*. Typhimurium agglutinins that play a role in adherence to animal tissue (Latasa *et al*., 2005; Morgan *et al*., 2007). Overexpression of BapA produces a very thick pellicle of cells at the liquid–air interface and is thought to be required for the cell–cell interactions that form such an aggregate (Latasa *et al*., 2005). The same study found that deletion of *bapA* significantly reduces the level of intracellular colonization in a mouse model, but does not contribute to invasiveness itself. This suggests a role for BapA during the initial stages of infection, which are dependent on adherence rather than invasion. Similarly, SiiE was shown to play a significant role in adherence to bovine enterocytes that as a result, impacted on the host immune response. However, the effect was relatively small, indicating that other factors may also be involved in adherence (Morgan *et al*., 2007). Using the RBH approach we found that SiiE has putative orthologues in a number of different plant-associated bacteria, including *Burkholderia cepacia* AdhA and two proteins of unknown function in *P. atrosepticum* (unpublished data). This led us to question whether SiiE may play a generic role in adherence in *S*. Typhimurium, binding to a range of biotic surfaces, including plants. Deletion of SiiE resulted in a decrease in adherence to plant roots, although the overall effect was relatively small (unpublished data), consistent with the observed result in animal tissue. Whether BapA is involved in adherence to plant material remains to be tested, although it may contribute to building a microbial community that forms on plant surfaces.

Agglutinins are multirepeat proteins and family members share little overall sequence identity except in a conserved consensus motif. For example, *siiE* has only 46% nucleotide identity to a homologue in *P. atrosepticum*, but 14% amino acid identity, below the accepted levels of homology. Despite the differences in their primary sequence, agglutinins show considerable similarity of structure and function. *Pectobacterium atrosepticum* is a phytopathogen, but is capable of inducing the haemagglutination of erythrocytes from a number of different species, as well as adherence to plant tissue (Wallace & Perombelon, 1992).

Agglutinins have been reported in a number of plant-associated bacteria and several have an established role in biofilm formation. The *lap* cluster in *Pseudomonas fluorescens* encodes a large adhesin protein (LapA) required for biofilm formation (Hinsa *et al*., 2003). This cluster is sensitive to environmental stimuli in that levels of inorganic phosphate are sensed by PhoB, which in turn regulates secretion of LapA (Monds *et al*., 2007). Another large agglutinin-like protein, HecA, has been shown to play a more specific role in bacteria–plant interactions. HecA is encoded by *Dickeya* spp. and *P. atrosepticum*, and is homologous to the *Bordetella pertussis* filamentous haemagglutinin FHA (Stibitz *et al*., 1988). A gene with homology to FHA

on the *K. pneumoniae* genome has also been indicated on Fig. 1. In *Dickeya* spp., a mutation in *hecA* resulted in a marked reduction in virulence on *Nicotiana clevelandii* leaves, which was restored by complementation. Necrosis of leaf epidermal tissue underlying the bacterial colony occurred with wild-type bacteria, but not with the *hecA* mutant. HecA was also shown to be required for cell aggregation on leaf surfaces, with HecA-dependent adherence and virulence being specific for *N. clevelandii* (phenotypes did not occur on the leaves of other *Nicotiana* species or on potato tuber slices or chicory leaves) (Rojas *et al.*, 2002). If its role in adherence is not directly responsible for its effect on virulence, HecA may have two functions: one general function in cell aggregation and a second specific function in virulence. Whether the agglutinin-like proteins in animal-associated pathogenic bacteria also have host-specific functions in the context of bacterial plant interactions, in adherence, virulence or both remains to be addressed.

Cellulose is an important component of the biofilm matrix and *S.* Enteritidis mutants deficient in cellulose synthase genes were found to be unable to form a biofilm under a variety of conditions (Solano *et al.*, 2002). The observed lack of cellulose production did not affect bacterial virulence in a number of different animal infection studies, showing that it is not required for animal host infection. However, cellulose production may be important in biofilm formation in association with plant hosts (Matthysse *et al.*, 2008) and it appears that the complex, multicomponent nature of the biofilm matrix may be required for mediation of bacteria–plant interactions.

A screen of *S. enterica* serovar Newport mutants that were deficient in binding to alfalfa shoots identified a role for AgfD, a key regulator of biofilm constituents (Barak *et al.*, 2005). AgfD regulates the expression of curli, which are thin, coiled fimbriae-like proteinaceous fibres that mediate cell–cell interactions in biofilms and binding to animal cell surfaces (Kikuchi *et al.*, 2005). Deletion of *agfB* (surface-exposed curli nucleator) reduced binding to alfalfa shoots, but this effect was only observed during initial adherence and was diminished after 24 h (Barak *et al.*, 2005). Furthermore, deletion of the curli subunit *agfA* did not affect binding capacity. However, in addition to regulating expression of curli genes, AgfD also regulates the expression of cellulose, which may explain why the loss of curli synthesis alone elicited only a partial effect. Further studies have found that curli alone do not appear to contribute to binding of plant material. Deletion of *S.* Typhimurium CsgD (AgfD equivalent), which rendered the bacteria unable to form biofilms or express curli, did not affect binding to alfalfa sprouts or seed coats (Torres *et al.*, 2005). Similarly, deletion of *E. coli* O157:H7 curli genes alone did not abrogate binding to either lettuce leaves (Boyer *et al.*, 2007) or alfalfa sprouts (Jonas *et al.*, 2007). The regulatory interactions defining the matrix components are complex and will require further work to disentangle. For example CsgD, which regulates expression of curli and cellulose, also regulates expression of the agglutinin BapA (Matthysse *et al.*, 2008).

Polysaccharides other than cellulose are also found in biofilm matrices, and are required for successful bacterial colonization of plant hosts. Poly-β-1,6-*N*-acetyl-D-glucosamine (PGA) was recently shown to be essential for *E. coli* binding to alfalfa sprouts. However, maximal binding only occurred in combination with cellulose and colonic acid production in addition to PGA (Matthysse *et al.*, 2008). Other factors as yet unidentified, are also known to be involved, as implied by an *rpoS* mutant in *S. enterica* that was defective in initial binding to alfalfa sprouts (Barak *et al.*, 2005). RpoS is an alternative sigma subunit of RNA polymerase and is known to regulate both curli and cellulose production via *agfD* expression. However, complementation of this particular mutant with plasmid-borne *rpoS* did not restore either curli or cellulose production, although it did restore adherence to alfalfa sprouts, suggesting that factors other than curli and cellulose are required for binding (Barak *et al.*, 2005). This result also indicates that timing and coexpression of these factors are likely to be significant in the regulation of bacteria–host interactions.

The carriage of genes required for biofilm formation associated with plant interactions is not universal. For example, the gut commensal strain *E. coli* K-12 does not encode determinants for PGA or cellulose. However, homologous genes for PGA and cellulose synthesis are found in a variety of enterobacteria including *K. pneumoniae* (two clusters of putative cellulose synthesis genes are indicated in Fig. 1, labelled CEL), and also in more distantly related species of both animal- and plant-associated bacteria. This likely reflects the wide requirement of biofilm formation by a number of different species and the use of similar biological strategies for biofilm production.

Invasion

The mechanisms by which enteric pathogens invade plant tissues following adherence are largely unknown, in contrast to invasion of animal hosts. Invasion in the context of plant hosts normally refers to internalized bacteria that may be either extra- or intracellular, whereas invasion of animal hosts usually refers only to intracellular entry. The invasion of plants by human pathogenic enterobacteria appears to be largely extracellular, whereby bacteria reside within the fluid-filled apoplastic spaces between cells. This is similar to the mode of invasion seen with *P. atrosepticum* and *Dickeya* spp., which also invade the apoplast, but do not appear to internalize within plant cells (Toth *et al.*, 2003).

Most studies of this nature on human pathogenic enterobacteria have been carried out using either fluorescently or bioluminescently labelled bacteria. A common technique involves sterilizing the external surfaces of plant tissue, allowing quantification of bacteria that are internalized and protected from the sterilizing agent. The majority of these studies have shown that animal pathogenic enterobacteria preferentially invade plant root tissue rather than foliage. For example, there are comprehensive studies that indicate the presence of internalized bacteria solely in roots and not in leaves, irrespective of plant growth conditions and inoculation procedures (Cooley *et al.*, 2003; Jablasone *et al.*, 2005). In addition, bacterial invasion has been imaged at lateral root junctions, perhaps because extracellular spaces at these points are large and amenable to bacterial entry (Cooley *et al.*, 2003; Dong *et al.*, 2003a, b; Warriner *et al.*, 2003). Some bacteria possess the ability to invade plant cells once they have access to the apoplast. Colonization of barley root by *S.* Typhimurium was shown to be greatest on root surfaces, but bacteria were also detected in rhizodermal cells adjacent to colonies external to the plant 2 weeks postinfection (Kutter *et al.*, 2006). Using FISH, they showed that the bacteria were also present in microcolonies within the root cortex 3 weeks postinfection. The same study compared the colonization capacity of *Listeria* strains, none of which exhibited endophytic colonization. The ability of *Salmonella* to penetrate plant cells has also been demonstrated in *A. thaliana*. Fluorescently marked *S.* Typhimurium cells were found to invade root hairs within 3 h of infection and proliferation of motile bacteria within rhizodermal cells was observed after 20 h (Schikora *et al.*, 2008). Again, colonization of foliage was found to be less extensive than root colonization and bacteria that were artificially internalized into the leaves did not appear to spread systemically from the point of infiltration. However, bacteria could be detected in newly formed leaves 1 month after introduction (Schikora *et al.*, 2008).

Motility is a requirement for migration on plants, both on external surfaces and within plant tissue. *Salmonella enterica* serovar Typhimurium mutants deficient in flagella synthesis and motility were unable to invade *A. thaliana* at lateral root junctions, unlike their wild-type counterparts (Cooley *et al.*, 2003). It was speculated that the lack of internalization may have been due to an inability of the mutants to locate to entry points at the lateral root junctions, and that a higher initial inoculum may overcome this deficiency. However, an increase in the inoculum of 100-fold had no effect on the levels of internalized bacteria. While these studies clearly demonstrate a requirement for flagella in plant colonization, other contributing mechanisms also exist. Plants represent an important host reservoir for *K. pneumoniae* and high levels of endophytic colonization have been demonstrated with a variety of different monocot and dicot species (Dong *et al.*, 2003a, b). As is the case for other human pathogenic enterobacteria, *K. pneumoniae* appears to invade plant tissue at lateral root junctions (Dong *et al.*, 2003a, b), even though it is aflagellate. How colonization is achieved is so far unknown, although it is likely to be dependent on whether the bacteria are part of a biofilm or growing in a planktonic state. Elucidating the mechanism of internalization used by *K. pneumoniae* may have important implications for other human pathogenic enterobacteria, which are also aflagellate, for example some VTEC isolates.

Invasion of plant tissue extends beyond roots for some systems of human pathogenic bacteria–plant interactions. These bacteria have been detected within the tissue of a variety of different fruiting bodies. For example, a number of *S. enterica* serovars isolated from diverse sources were tested for their ability to colonize tomato fruits at different stages of growth, fruit ripening and storage (Shi *et al.*, 2007). In general, all serovars that were tested internalized within fruits and were recovered at *c.* 1% of the original starting population after overnight infection. This capacity to internalize and persist appears to be serovar dependent. Moreover, some *S. enterica* serovars were detected within the tissue of 6–7-week-old tomato fruits, following inoculation of flowering parts (Shi *et al.*, 2007). Although observed levels of detectable bacteria were low, this pivotal finding suggests that vertical transmission of pathogenic bacteria, from one plant generation to the next, is possible.

Marketplace studies have reported the presence of human pathogenic enterobacteria on a variety of fresh produce (Wells & Butterfield, 1997). The presence of these bacteria has usually been accounted for as a result of contamination of mature fruits, and more so if the fruit had been damaged. Alternatively, bacteria may be present as a result of successful colonization at an earlier stage, for example during fruit development. One study found that both *E. coli* O157:H7 and *S. enterica* serovar Hartford were capable of significant growth following artificial internalization into orange fruits. In addition, both species were able to exploit damaged fruits and internalize into artificially wounded orange fruits (Eblen *et al.*, 2004). The acidic environment found in citrus fruit presents a low pH-stress to the bacteria. It is known that a number of enterobacteria, including *Salmonella* and *E. coli*, are able to adapt to a mild acid stress that subsequently provides protection against a much higher acid shock, comparable to the conditions of the human gut (Foster, 1991). Acetic acid has been shown to induce an acid tolerance response in *S.* Typhimurium at 20 °C (Greenacre *et al.*, 2003), as well as to provide cross-protection against other stresses (Greenacre & Brocklehurst, 2006). This implies that the colonization of citrus fruit may in fact enhance survival of salmonellae in transit through the human digestive tract.

Establishment

Internalization within plant hosts provides access to nutrients and limits the number of competing species likely to be present, especially those within the rhizosphere. In order to establish internal colonization, the bacteria need to avoid or suppress the plant immune response. In plants, there are two distinct steps to microorganism recognition. In the first step, generic pathogen- or microorganism-associated molecular patterns (PAMPs/MAMPs) such as flagella are detected by membrane-bound receptors resulting in PAMP-triggered immunity (PTI). The second step involves recognition of specific pathogen effector proteins by plant resistance proteins, on a gene-for-gene basis, which results in effector-triggered immunity (ETI) (Jones & Takemoto, 2004; Jones & Dangl, 2006). The similarities in pathogen recognition by plants and animals is not limited to the functional level, as plant resistance proteins contain a similar leucine-rich domain to that present in Toll-like receptors (Caplan *et al.*, 2008). Induction of ETI often results in the hypersensitive response, a form of localized programmed cell death that limits systemic spread of the pathogen. Successful pathogens evade or suppress ETI, when, for example, effector molecules go undetected or manipulate the host defence response, which results in effector-triggered susceptibility (ETS).

Much of the work on plant immune responses has been carried out in archetypal host–pathogen systems, such as infection of *A. thaliana* with *P. syringae*. Whether internalized human pathogenic enterobacteria can evade or manipulate the host response is largely unknown, although different reports suggest that PTI is induced, and that some effector proteins are also recognized. A relatively short-term infection of *A. thaliana* with *E. coli* O157:H7 did not result in disease symptoms or support bacterial growth, but did stimulate plant genes characteristic of PTI (Thilmony *et al.*, 2006). In contrast, infection with wild-type *P. syringae* pv. *tomato* resulted in visible disease symptoms within 24 h and supported bacterial replication (Thilmony *et al.*, 2006). While both bacteria induced a number of PAMP genes, the *A. thaliana* expression profile induced by *E. coli* O157:H7 correlated highly with that of *P. syringae* pv. *tomato* derivatives that were mutated for pathogenic effector genes. This suggests that human pathogenic enterobacteria induce PTI, whereas phytopathogenic effectors are able to manipulate the response by repressing expression of many PAMP-induced genes (i.e. induce ETS). This study did not find a strong flagellin-dependent response, which indicates that bacterial flagellin did not substantially contribute to the PAMP-induced response. These findings at first seem to be at odds with the paradigm that bacterial flagellin is a key elicitor of PAMP recognition. The authors suggest that this discrepancy may be due to redundancy in PAMP recognition, where recognition of additional patterns such as lipopolysaccharide, may trigger a response that overlaps with flagellin perception.

Although only a basal PTI response was raised by *A. thaliana* following inoculation of *E. coli* O157:H7, it appears that when exposed to animal pathogenic bacteria, plants may be able to detect effector proteins that are required for infection of animal hosts. Animal pathogenic enterobacteria that invade animal host cells, including *Salmonella*, *Shigella* and *Vibrio* spp., use the TTSS to deliver effector proteins into host cells in a manner similar to plant pathogenic bacteria. *Salmonella* strains encode two TTSS, located on *Salmonella* pathogenicity islands (SPI) 1 and 2. TTSS SPI-1 is required for initial invasion of cells, such as macrophages, while TTSS SPI-2 maintains the intracellular vacuole that protects the growing bacterial colony (Ruiz-Albert *et al.*, 2002; Steele-Mortimer *et al.*, 2002). It is well established that effector molecules secreted by phytopathogens are detected by host plant cells, and induce an immune response (Jones & Dangl, 2006). However, components of *Salmonella* SPI-1 also appear to be recognized by host plants. For example, deletion of *spaS*, which encodes the structural subunit of the TTSS SPI-1 apparatus, and *sipB*, an effector protein and translocator, resulted in increased colonization of alfalfa roots and wheat seedlings (Iniguez *et al.*, 2005). Interestingly, *K. pneumoniae* isolates do not encode a TTSS (or flagella genes), which may be a reason why the host response is not triggered by this species. In contrast, plant pathogenic bacteria such as *P. syringae* secrete effector proteins via the TTSS that trigger a hypersensitive response (Collmer *et al.*, 2000). The lack of virulence factors may go some way to explain why *K. pneumoniae* strains are such successful plant colonizers (Iniguez *et al.*, 2005).

While the majority of studies have reported asymptomatic colonization of plants by animal pathogenic enterobacteria, this is not always the case. *Salmonella enterica* serovar Typhimurium introduced into *A. thaliana* leaves, either by vacuum infiltration or by whole plant dipping, resulted in chlorosis and wilting of leaves, 2 weeks post-inoculation (Schikora *et al.*, 2008). The ability of a microorganism to act as a pathogen on hosts from different kingdoms is not without precedent. For example, virulence factors from *Pseudomonas aeruginosa* were shown to elicit disease both in *A. thaliana* and in rodents (Rahme *et al.*, 2000).

Recognition of pathogens by both animal and plant cells triggers a cascade of reactions that results in activation of the host defence response. In both systems, the type of response triggered is dependent on the pathogen and its effectors. For example, biotrophic and necrotrophic plant pathogens elicit distinct responses in plant cells, dependent on which hormone pathways are triggered. The presence of biotrophic pathogens normally induces ETI via the salicylic acid (SA)

signalling pathway, whereas necrotrophic pathogens mainly induce the jasmonic acid (JA)/ethylene pathway. Infection of an *A. thaliana* cell suspension (protoplasts) with *S.* Typhimurium resulted in an increase in expression of two mitogen-acitvated protein kinases (MPK3 and MPK6) that have been implicated in the PTI response (Schikora *et al.*, 2008). In particular, both kinases are part of the signalling cascade that responds to bacterial flagellin (Asai *et al.*, 2002). Further experiments showed that MPK6-deficient plants were less resistant to the bacteria and supported higher numbers of bacteria than wild-type plants. Interestingly, this study also found that the JA-signalling pathway appear to be particularly important for resistance to *S.* Typhimurium, which suggests that the bacteria are perceived as necrotrophs. Iniguez *et al.* (2005) found that both SA and ethylene pathways were triggered in *A. thaliana* by the presence of *S.* Typhimurium, but that expression of the SPI-1 effector triggered just the SA pathway. More work is required to further investigate the host response triggered by human pathogenic enterobacteria and to determine the extent of symptomatic plant disease that they can cause.

Colonization of plants by bacteria, whether internalized or not, necessitates utilization of plant-derived nutrients. Genomic comparisons have highlighted key factors in the genomes of animal-associated enterobacteria, which are more commonly found among plant-associated bacteria. RBH analysis of *K. pneumoniae* to plant-associated bacteria highlighted the presence of six PTS that indicate transport and catabolism of plant-derived carbohydrates (Fig. 1), including cellobiose and fructose. Plant-associated RBH to genes present in the *S.* Typhimurium and VTEC genomes include several dehydrogenases (Toth *et al.*, 2006 and unpublished). Dehydrogenases are necessary for catabolism of a variety of carbon sources and their presence mostly in plant-associated bacteria suggests a role in nutrient acquisition from plants. That these enzymes have putative orthologues in animal pathogenic enterobacteria may indicate an ability to exploit similar biochemical sources and may be a component of adaptation to a plant environment. From genomic comparisons, five dehydrogenases were detected in the *S.* Typhimurium genome and six in the VTEC genome (Sakai isolate) with putative orthologues in plant-associated bacteria. Examples in the VTEC genome include a xanthine dehydrogenase, required for the catalysis of basic reactions in the C, N and S cycles. This enzyme is in a three-gene cluster, which includes a putative molybdenum nitrogenase that contains an iron sulphate-binding domain characteristic of cofactors that catalyse the reduction of N_2 to ammonia. The cluster appears to be widespread among plant-associated bacteria, but is not generally found in animal-associated bacteria. The homologous cluster in *P. fluorescens* shares *c.* 49% identity at the DNA level and the CDS share 56–71% amino acid identity. The association of these particular enzymes with plant-associated bacteria suggests a role related to the plant environment. A preliminary comparison with animal-associated bacteria has shown the presence of the same cluster only in *Enterobacter sakazakii*, a species normally associated with mammalian gut flora, but that is also widespread in the environment (Lehner & Stephan, 2004; Kim *et al.*, 2006).

The establishment of plant colonization is also affected by competition with the resident microbial community. Competition has been demonstrated between animal pathogenic enterobacteria and *Enterobacter asburiae*, another member of the *Enterobacteriaceae* that is frequently isolated from *A. thaliana* and lettuce plants. Growth of both *S. enterica* serovar Newport and *E. coli* O157:H7 on *A. thaliana* roots was inhibited by up to two orders of magnitude by *E. asburiae* (Cooley *et al.*, 2003). It was shown that inhibition was mediated by a diffusible factor and that both bacterial species were in competition for the same carbon sources present in lettuce seedling exudates (Cooley *et al.*, 2006). In contrast, the presence of some resident microbial community may support growth of pathogenic enterobacteria. *Wausteria paucula* (formerly *Ralstonia paucula*) is an epiphyte prevalent on *A. thaliana* and lettuce plants. The presence of *W. paucula* was found to enhance growth of *E. coli* O157:H7 on sterile-grown lettuce roots, although the basis of this interaction is unknown (Cooley *et al.*, 2006). Investigation of several carbon sources present in plant exudates showed that there was no overlap in nutritional requirements between both species, in contrast to the situation found for competing species. It is possible that the presence of *W. paucula* resulted in a release of nutrients not normally available to *E. coli* O157:H7. This idea is supported by studies that indicate an apparently mutualistic relationship between plant-associated bacteria that are efficient at degrading plant cell wall material and human pathogenic enterobacteria. For example, coincubation of *S.* Typhimurium and *P. atrosepticum* on potato, carrot or pepper discs resulted in a 10-fold increase in the *S.* Typhimurium population, compared with incubation of *S.* Typhimurium alone (Wells & Butterfield, 1997). A similar but less-pronounced effect was observed following coinoculation of *S.* Typhimurium with pectolytic *P. fluorescens* or *Pseudomonas viridiflava* (Wells & Butterfield, 1997).

Concluding remarks and future direction

The bacterial family *Enterobacteriaceae* is one of the most studied groups of organisms, largely due to the importance of many of its members as devastating pathogens of animals and plants, as well as the use of *E. coli* as a model microorganism and laboratory 'work-horse'. By considering the family as a cohesive unit, rather than focusing on individual pathogens and their most apparent hosts, it is

possible to see that they comprise a diverse collection of related bacteria that are capable of persisting in a broad range of environments. This is in part, due to the transfer of genetic information within the family and with other microorganisms in different environments. Their evolutionary history appears to have provided an ability to colonize a wider host range where it has proven necessary for survival and spread, although there are distinct adaptations towards specific niches for the majority. However, it is apparent that there is a continuum of available niches across biological kingdoms that can be colonized to a greater or a lesser extent.

There is considerable evidence to support the hypothesis that plants are used as alternative hosts by animal pathogenic enterobacteria, although many questions remain unanswered regarding the mechanisms of plant colonization by these organisms. For example, the genome sequences of key animal pathogenic enterobacteria have been available for several years, yet a high proportion of genes with unknown biochemical function remain. Genomic comparisons are only one way to approach a definition of the plant-associated functions of some of these genes and supported by the appropriate molecular techniques may yet elucidate mechanisms for plant colonization. Traditional genetic approaches that assign a phenotype to a genotype have already yielded a great deal of information on bacterial physiology. However, the picture is less clear when multiple factors are involved in the phenotype, for example with bacterial biofilms. The challenge for future work will be to examine the expression profiles of various factors *in situ* in order to disentangle the complexity, function and dynamism of these living microbial communities.

A key goal for future research must be to better understand the interactions between animal-associated bacteria and host plants. Pan-genome analyses are, and will continue to be, among the most valuable tools for studying host–microorganism interactions at the level of the entire system, rather than as a collection of independent parts. This and other systems biology approaches can be extended to identify and elucidate regulatory networks in the interactions. An immediate and tangible benefit of genomics work has been to bring together scientists across disciplines, including those that work with divergent bacteria–host systems. One consequence of focusing on a single host system, either plant or animal, has been that the nature of the organism in question, in terms of the complete scope of its possible interactions, has been restricted in consideration. Only now are we beginning to share expertise from a range of research areas to help in our understanding of these microorganism–host partnerships. This is a fascinating area that will broaden our appreciation of animal pathogenic enterobacteria outside their 'traditional' hosts, and will shed light on the transmission of the pathogens from host to host and through the food chain, bringing value to the wider community.

Acknowledgements

N.H., L.P. and I.T. are supported by Scottish Government Rural and Environmental Research and Analysis Directorate. N.H. and L.P. carried out the unpublished work. Our thanks go to P. Birch and E. Gilroy for critical reading of the manuscript.

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