

Methods to investigate interactions between human pathogenic enterobacteria and plant hosts

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Introduction

Generalist bacteria from the enterobacteria family are able to adapt to a variety of environments and have been isolated from hosts across different kingdoms. Some, such as verotoxigenic *Escherichia coli* and *Salmonella* species are commonly associated with animal hosts, but

there is good evidence to show that these bacteria can also colonise plants. Fresh produce is now recognised as an important vehicle of transmission of these bacteria (1-3). Plant material represents more than inert surface to transport the bacteria into the food chain, instead the

bacteria interact with plants and are able to utilise them as alternative hosts (4-7).

The aim of our work is to elucidate the interactions between human pathogenic enterobacteria (HPE) and plants. We use a two pronged

approach, combining bioinformatics and molecular biology to yield information on the genetic basis to the interactions. The over-arching goal of the work is to limit transmission of pathogenic bacteria through the food chain by understanding how they colonise alternative host species.

Results

HPE have the ability to adhere to (Fig. 1) and colonise (Fig. 2) both model plant species and leafy vegetables. *Nicotiana benthamiana* was used as a model plant species to assess the level of bacteria that closely associate with the roots. Both *E. coli* O157:H7 and *Salmonella enterica* serovar Typhimurium were recovered in significantly higher numbers than *E. coli* K-12 (Fig. 2). In addition, the level of bacteria recovered was equivalent or more than that of the endophyte *Klebsiella pneumoniae* (strain Kpn 342).

Figure 1. (below)
S. Typhimurium adhere to spinach root hairs. Con-focal micrograph of GFP-labelled bacteria and *Spinacia oleracea* roots. The bacteria were incubated with roots for two hours at 18°C.

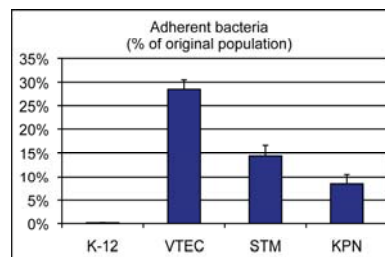
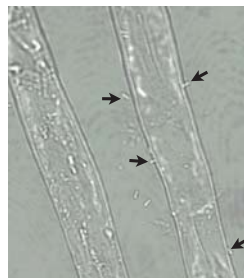


Figure 2. (above)
HPE adhere to *N. benthamiana* roots. The graph shows the mean number of bacteria recovered from roots, expressed as a percentage of the original population. Key: K-12 is *E. coli* MG1655; VTEC is *E. coli* Sakai; STM is *S. Typhimurium*; KPN is *K. pneumoniae*. Approximately 1×10^7 cfu/ml bacteria were added to the roots and enumerated after 2 hours at 18°C by plating. Error bars refer to the standard error of the mean.

Genomic comparisons have been carried out between HPE and bacteria that are normally associated with plants to search for genetic determinants that may be involved in plant colonisation. A reciprocal best hit (RBH) approach was used to ensure robust homology matches. Coding sequences that make RBH to sequences in plant-associated bacteria but not in other animal-associated bacteria are indicated on the chromosomes of *E. coli* O157:H7 (Sakai isolate) (Fig 3A) and *S. Typhimurium* (Fig 3B), using a program developed in-house, GenomeDiagram (8). RBH tend to be clustered together in regions of horizontal transfer, many are phage-derived and a good proportion have as yet, undefined functions.

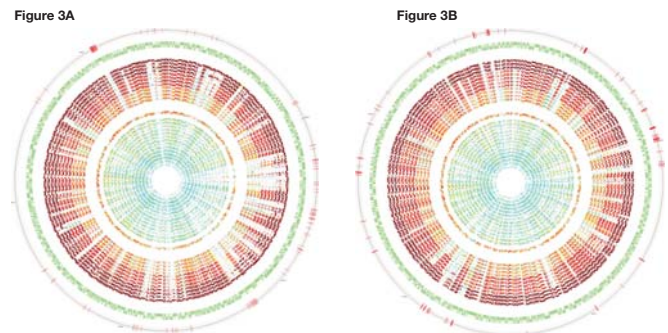


Figure 3. Genomic comparisons show coding sequences shared between HPE and plant-associated bacteria. GenomeDiagrams of *E. coli* O157:H7 (A) and *S. Typhimurium* (B) and comparators. Coding sequences of the query chromosome are in green on the outer ring. Matches with comparator sequences are in the inner rings. RBH to sequences in plant-associated and not animal-associated bacteria are indicated by red marks on the query sequence. The degree of homology is colour-coded where dark red shows the highest level and light blue the lowest. See (10) for details.

An example of genes that are shared between HPE and several plant-associated bacteria is the agglutinin-like family. Agglutinins mediate host binding and bacteria cell-cell interactions. *S. Typhimurium* encodes an agglutinin (SiiE) that plays a role in adherence to bovine enterocytes (9). Deletion of *siiE* and *siiF* (a component of the type 1 secretion system) resulted in a decrease in adherence of *S. Typhimurium* to *N. benthamiana* roots (Fig 4). However, the effect was small indicating that other factors are also involved. Work is on-going to further characterise the role of SiiE and other RBH, in colonisation of plants.

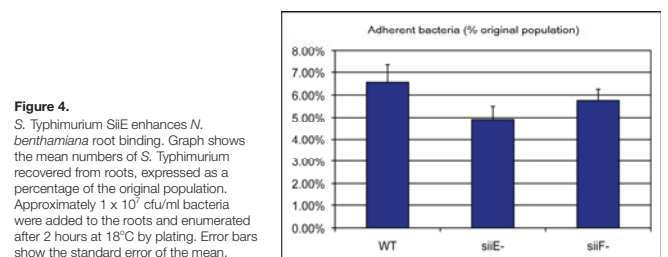


Figure 4. *S. Typhimurium* SiiE enhances *N. benthamiana* root binding. Graph shows the mean numbers of *S. Typhimurium* recovered from roots, expressed as a percentage of the original population. Approximately 1×10^7 cfu/ml bacteria were added to the roots and enumerated after 2 hours at 18°C by plating. Error bars show the standard error of the mean.

Conclusions

Human pathogenic enterobacteria are able to adhere to and colonise plants. Bioinformatics techniques allow genomes to be screened to find coding sequences that are also present in plant-associated bacteria and as such, may play a role in plant colonisation. Together with molecular biology approaches we aim to determine the genetic basis for the interactions between human pathogenic bacteria and plants.

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