Internalisation of human pathogenic enterobacteria into edible vegetables.

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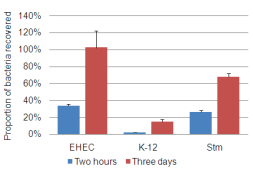
Background

Enterohaemorrhagic *Escherichia coli* and *Salmonella enterica* are considered two of the most serious food-borne bacteria. These pathogens are able to adapt to a wide variety of environments and can enter the food chain at any point. Over recent time there have been a number of notable, large-scale outbreaks associated with fresh fruit and vegetables. Both pathogens are zoonoses and commonly associated with animals, but we have proposed that EHEC and *S. enterica* can colonise plants and use them as alternative hosts (Holden *et al.*, 2009).

Results

Bacteria-plant interactions were characterised for plants implicated in recent food-borne outbreaks infected by EHEC and *S. enterica* serovar Typhimurium. Infection was initiated in the root system since growth of zoonotic pathogens has been shown to occur to higher levels in the rhizosphere compared to the phyllosphere (Brandl *et al.*, 2004; Cooley *et al.*, 2003). We assessed the interactions of EHEC and *S.* Typhimurium with tomato roots over two hours and three days. Bacteria were recovered in significantly higher numbers after three days of infection compared to two hours, showing bacterial proliferation on tomato roots. In addition, far higher numbers of both EHEC and *S.* Typhimurium were recovered in comparison to the gut commensal *E. coli* K-12 (Fig. 1) at both time points. Similar data has been obtained for association with lettuce and spinach.

Figure 1 Bacteria in association with tomato roots. EHEC, *E. coli* K-12 or *S.* Typhimurium (Stm) were recovered from the root system after two hours or three days of infection and the numbers expressed as a proportion of the inoculum used for the initial infection.



We have found that the pathogens are effective in migrating from the roots to foliar tissue and EHEC and *S.* Typhimurium can be commonly isolated from the leaves of plants grown in hydroponics. However, it is important to distinguish whether the bacteria are on the external surfaces, or within the internal plant tissue, since current production methods only remove surface-associated microbes. Internalised EHEC and *S.* Typhimurium were found to be present in different plant tissues of lettuce and tomato, in particular in the crown (root-shoot junction) and leaves (Fig. 2). Microscopic examination showed EHEC migration into the root cortex apparently via the apoplast (Fig. 3), and some cells can be found within xylem vessels (Fig. 4).

Figure 4 Bacteria in epidermal tissue and in xylem. The micrographs show a transverse section of lettuce root (blue) infected with EHEC (green) (A) and a close-up on the xylem vessels (B). The scale bars are 50 µm (A) and 10 µm (B).

Figure 2 Bacterial internalisation into different plant tissues from root infections. Lettuce seedlings infected with EHEC were surface sterilised with gentamicin and bacteria detected from dissected tissue. The graph shows the proportion of replicate plants that contain internalised bacteria from different tissues, on different days.

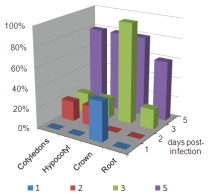
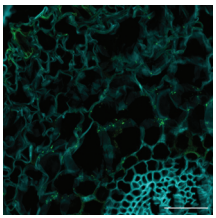
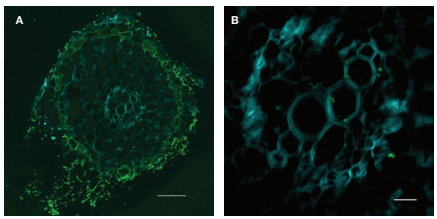


Figure 3 Bacteria within root cortex. The micrograph shows a transverse section of spinach crown (blue) infected with EHEC (green). The scale bar is 50 µm.





These experiments show that EHEC and *S.* Typhimurium can adhere to and proliferate on the roots of vegetables from where they have the ability to internalise into plant tissue. They can also be detected from internal edible plant tissue.

References

Brandl, M. T., Haxo, A. F., Bates, A. H. & Mandrell, R. E. (2004). Comparison of survival of *Campylobacter jejuni* in the phyllosphere with that in the rhizosphere of spinach and radish plants. *Appl Environ Microbiol* **70**, 1182-1189.

Cooley, M. B., Miller, W. G. & Mandrell, R. E. (2003). Colonization of *Arabidopsis thaliana* with *Salmonella enterica* and enterohemorrhagic *Escherichia coli* O157:H7 and competition by *Enterobacter asburiae*. *Appl Environ Microbiol* **69**, 4915-4926.

Holden, N., Pritchard, L. & Toth, I. (2009). Colonization outwith the colon: plants as an alternative environmental reservoir for human pathogenic enterobacteria. *FEMS Microbiol Rev* **33**, 689-703.