Association of *Escherichia coli* O157:H7 and Fresh Produce

PhD on Transcriptome Analysis of Enterohaemmorhagic *Escherichia coli* O157:H7 *in planta*

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Introduction

Enterohaemmorhagic *Escherichia coli* O157:H7 is a human pathogen which can be transmitted to humans via the ingestion of contaminated fresh produce.

Many studies have looked at the transcriptome of *E. coli* O157:H7 in association with leafy tissue. However, there is much higher colonisation potential in the rhizosphere compared to the phyllosphere, due to nutrient and water availability, and less UV. Furthermore, previous transcriptomic work has been carried out on post-harvest, processed plant material[1], for which there will be quite different plant-microbe interactions.

My project aims to determine the transcriptome of *E. coli* O157:H7 when exposed to living plant roots, to understand how this bacterial species interacts with and colonizes plants.

Aims

-To examine the bacterial genes of *E. coli* O157:H7 that are differentially regulated when exposed to the roots of living plants, specifically domesticated and wild lettuce, spinach and pea using microarray technology.

-To examine the colonisation phenotypes of two strains of *E. coli* O157:H7 in these four plant species in the different plant environments.

E. coli O157:H7 needs to adapt to new environments before entering a human host and this project will determine the adaptive processes involved in plant colonisation.

Methods

RNA extraction methods are currently being optimised to obtain high quality and sufficient quantity of bacterial *m*RNA from infected plants for transcriptomics: the methods use commercial kits (Qiagen Rneasy) and traditional extractions (hot-phenol[2]). Optimisation is on-going for a number of *in vivo* and *in vitro* plant environments. Once sufficient quality RNA has been obtained, these will be converted to *c*DNA and hybridised to Agilent *E. coli* DNA microarrays.

Comparative colonisation assays have been set up for *E. coli* isolate Sakai (clinical isolate from white radish sprouts, *Stx*$^-$, Kan^R) and *E. coli* isolate TUV93-0 (bovine isolate EDL933 *Stx*$^-$ Na^R), in different plant environments, for 4 plant species. For each strain, colonisation is being tested in the phyllosphere (on the adaxial and abaxial sides of the leaves) and the roots/rhizosphere (from plants grown in compost and grown in hydroponics). Colonisation potential is measured from microbial counts on selective agar.

Results

Colonisation assays have shown differences between bacterial strains, plant niche and plant species. Inoculations of *E. coli* Sakai and TUV93-0 on the spinach phyllosphere resulted in higher levels of recovered bacteria (*have you done the stats to shown sig diffs?*) on the abaxial side in comparison to the adaxial side (Fig 1). In addition, *E. coli* Sakai was still detectable at high levels after 10 days, whereas *E. coli* TUV93-0 was below the limits of detection at this time point (Fig 1).

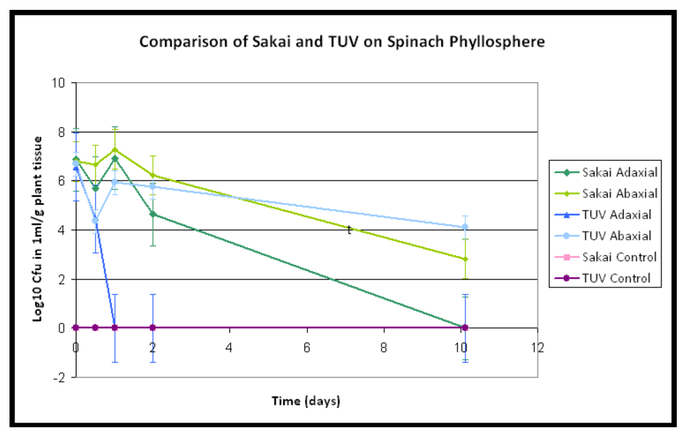

Figure 1. Colonisation of the Spinach Phyllosphere by *E.coli* O157:H7 strains Sakai and TUV over time.

Colonisation of the roots shows significantly higher levels of bacteria compared to the leaves and in some plant species, bacterial growth (Fig 2). After 10 days, the level of *E. coli* Sakai in wild lettuce (*Lactuca serriola*) has increased almost 10-fold from the inoculum level to 10^7 cfu/g plant tissue. The levels in spinach and round-head lettuce had decreased marginally from the initial inoculum.

These preliminary studies help to show the differences in colonisation within different areas of the plant by *E. coli* O157:H7 and help show the importance of the interaction with the rhizosphere.

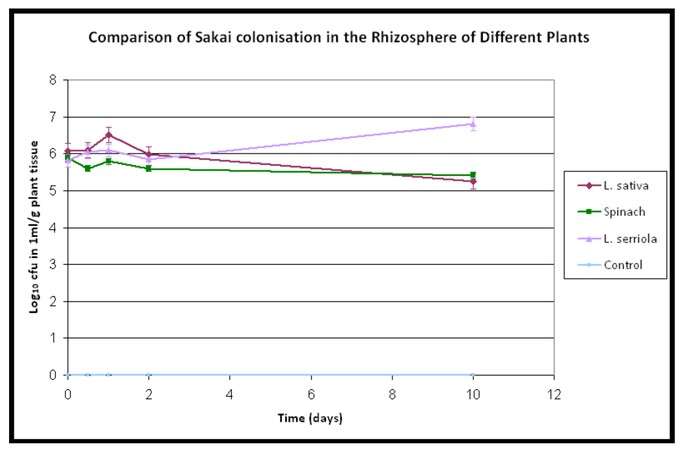

Figure 2. Colonisation of the roots of spinach, *L.sativa* (domesticated lettuce) and *L.serriola* (wild, prickly lettuce) by *E.coli* O157:H7 strain Sakai over time.

Conclusions

- There are marked differences in the colonisation potential of *E. coli* isolates Sakai and TUV93-0 within the phyllosphere and rhizosphere of different plants, for example, *E. coli* Sakai can colonise and establish a population on the roots of 3 of different plant species.
- Optimisation experiments of the RNA extractions is on-going, comparing yields from commercial kits (Qiagen), with traditional methods (hot phenol). This project will substantially extend previous work in the lab that successfully extracted sufficient bacterial *m*RNA for bacterial transcriptome analysis, for *E. coli* Sakai in association with spinach roots.

References:
1. Brandl, M. T. "Plant Lesions Promote the Rapid Multiplication of *Escherichia coli* O157:H7 on Postharvest Lettuce." Applied and Environmental Microbiology. 74.17 (2008): 5285-89
2. Schenk, A.; Weingart, H.; Ullrich, M. "Extraction of high-quality bacterial RNA from infected lead tissue for bacterial in planta gene expression analysis by multiplexed fluorescent Northern hybridization." Molecular Plant Pathology 9.2 (2008): 227-35.