Human pathogenic bacteria contamination of *Arabidopsis thaliana* seed following root inoculation

Michael B. Cooley*, William G. Miller and Robert E. Mandrell

Produce Safety and Microbiology Research Unit, USDA-ARS-WRRC, 800 Buchanan Street, Albany CA 94710, USA

Abstract

Enteric pathogens, such as *Salmonella enterica* and *Escherichia coli* O157:H7, have been shown to contaminate fresh produce. Several epidemics related to sprouts have been traced to contaminated seed. Under appropriate conditions these bacteria will grow on and invade the plant tissue. We have developed *Arabidopsis thaliana* (thale cress) as a model system with the intention of studying plant responses to human pathogens. Under sterile conditions and 100% humidity both pathogens grew to 10^9 CFU/gm on *A. thaliana* roots and to 5 X 10^6 CFU/gm on shoots. Furthermore, root inoculation leads to contamination of the entire plant, indicating that the pathogens are capable of moving on or within the plant. Inoculation with GFP-labeled *S. enterica* and *E. coli* O157:H7 showed invasion of the roots at lateral root junctions. Movement and, to some extent, invasion were eliminated using non-motile mutants of *S. enterica*. Inoculation of the seed before planting on soil also allowed growth of the pathogens on the foliage, though to a much lesser extent. Furthermore, contamination of the foliage declined as the plants matured and was undetectable at 30 days post germination. The incidence of contamination probably dropped due to exposure of the bacteria to reduced humidity and endogenous epiphytes from soil. Nevertheless, 15% of seed-pools harvested from these plants were found contaminated. The rate of recovery of Salmonella from seed was significantly less than recovery of E. coli O157:H7, Furthermore, the incidence of seed contamination from plants grown on un-sterile soil or soil supplemented with manure was similar to contamination levels of seed collected from plants grown in sterile soil. As such, *Salmonella enterica* and *Escherichia coli* O157:H7 are competent to colonize *A. thaliana* seed even with the selection pressure of epiphytic bacteria. Furthermore, contaminated seed were not sanitized by extensive washing and chlorine treatment, indicating that some of the bacteria reside in a protected niche on the surface of the seed or within the seed.

Introduction

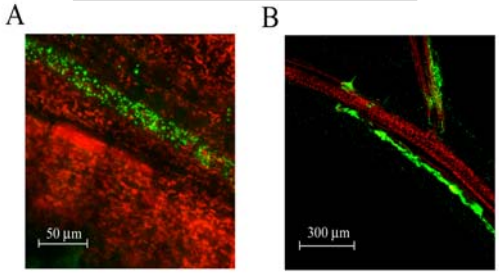
While the common reservoirs for *Salmonella enterica* and enterohemorrhagic *Escherichia coli* O157:H7 are animals, several outbreaks have been linked to fresh fruits and vegetables. The most likely source of contamination occurs post-harvest by cross-contamination from a variety of animal sources including humans. Nevertheless, it has been well documented that *S. enterica* and *E. coli* O157:H7 can survive and grow on fresh produce, especially on sprouts, where the contamination can exceed 10^7 CFU/gm fresh weight. Also, penetration of *E. coli* O157:H7 into plant tissue has been demonstrated, disinfection of the tissue is not a viable option. Hence, an understanding of *S. enterica* and *E. coli* O157:H7 colonization of plants would be valuable.

The plant biology involved in the interaction with human pathogens has not been investigated. We anticipate that some of the similarities between the interactions of plant pathogens and their hosts will extend to the seemingly novel interactions between human pathogens and plants. There is reason to assume that interactions occur frequently between human pathogenic bacteria and plants since enteric pathogens exist outside of their host organism for some portion of their life cycle. Remarkably, it has been shown that *Arabidopsis thaliana* makes a protein (FLS2, Flagellin Sensing) that can recognize a conserved region of bacterial flagellin. This flagellin region is conserved in many different types of bacteria including enteric pathogens such as *E. coli* O157:H7 and *S. enterica*. However, it is not well conserved in some symbiotic (*Rhizobium meliloti, Azospirillum brasilense*) and epiphytic bacteria (*Pseudomonas fluorescens*).

An understanding of specific interactions between human pathogens and plants will take considerable effort. This is especially true for the plant side of the interaction, since many crop plants have very large genomes and are poorly characterized genetically. Hence, we decided to create a model system with *A. thaliana*. The advanced genetics of *A. thaliana* should facilitate greatly the discovery of plant factors involved in these interactions. In this poster we demonstrate survival and growth of *S. enterica* and *E. coli* O157:H7 on both the roots and shoots of *A. thaliana*. More importantly, we show that inoculation of the pathogen at a single point on the plant eventually leads to contamination of the whole plant and that this contamination leads also to invasion of the plant and contamination of the seed.

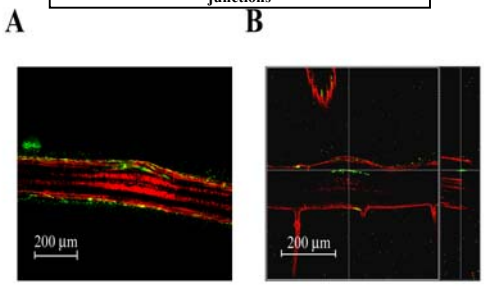
Plants were grown in vitro on slanted agar plates for inoculation and microscopy

GFP-labeled pathogens colonize the foliage and root



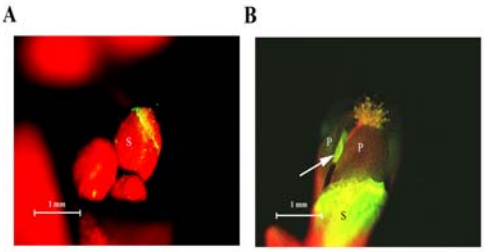
Plants are colonized while growing on agar slants (in vitro). Pathogens (green) often select niches on the leaf over a vein (A) and at lateral root junctions in the rhizosphere (B). Images are produced by confocal microscopy. Red is autofluorescence of the leaf and propidium iodide stain/autofluorescence of the root.

Pathogen invades the root tissue at lateral root junctions



Confocal microscopy of developing (A) and mature (B) lateral roots showing invasion of the pathogen (green). Plants were grown and inoculated on agar slants (in vitro). Invasion could be seen in approximately 20% of the lateral roots.

Pathogen colonizes the buds and flowers after root inoculation



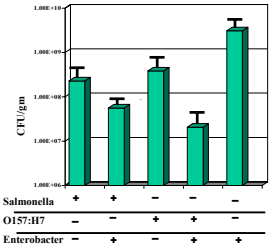
Plants growing on agar slants (in vitro) were inoculated on the roots 1.2 cm below the crown. The spread of the infection (green) led to colonization of the floral buds (A) and flowers (B). Arrow indicates an anther which autofluoresces green. S, sepal; P, petal.

Vortex, sonication and Cl_2 treatment did not sanitize the seed

	Unwashed seed		Washed seed			
	Number of pools	Number Positive	# of pools	# positive after vortex	# positive after sonication	# positive after Cl_2 treatment
Salmonella	143	18	10	10	8	5
O157:H7	127	34	8	8	8	3

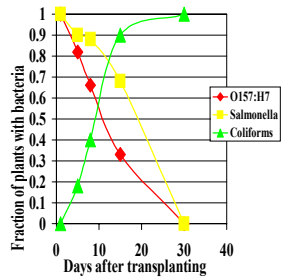
Seed samples (1000) were analyzed after germination by enrichment in lactose broth and selective plating on SS agar. Some of the contaminated pools were subsequently processed to sanitize the seed. After each sanitation step the seed was washed extensively with water and seed samples were taken to determine the effect of the treatment.

Pathogen growth on roots in vitro was reduced by competing bacteria



Plant grown on agar slants (in vitro) were inoculated with the indicated bacteria and sampled at 48 hr. *Enterobacter asburiae* (typical cotton endophyte) was isolated as an epiphyte from soil-grown Arabidopsis.

Detection of bacteria on leaves/flowers decreased after transplanting to soil



Bacteria was detected after enrichment by incubating flowers or cauline leaves for 24 hr in lactose broth followed by plating on SS agar (Salmonella and O157:H7) or chrome agar (coliforms). *Enterobacter asburiae* was frequently recovered, indicating that it is probably a major competitor.

Recovery of pathogen from seed was similar with plants grown in amended soils

Fraction seed pools with detectable pathogen

	Sterile soil	Un-sterile soil	Manure added
O157:H7	.21 (127)	.20 (20)	.22 (18)
Salmonella	.09 (143)	.11 (18)	.05 (18)

Seed from several plants were pooled, sampled (1000 seed) and germinated on M+S agar. Bacteria contaminating the seedlings was enriched by 24 hr incubation in lactose broth and plated on selective media. Several isolates were also verified by pulse field gel electrophoresis. The number in parenthesis is the number of seed pools analyzed.

Conclusions

- **Human pathogenic bacteria are capable of growth on and invasion of Arabidopsis in vitro.**
- **Growth of the pathogen leads to colonization of select niches on the plant, especially the roots, flowers and seed.**
- **Growth of the infected plants in soil suppresses the growth of human pathogenic bacteria, but did not eliminate the contamination of the seed.**
- **The inability to sanitize the seed indicated that some portion of the bacteria are tightly bound in a protected niche on the surface of the seed or are internal.**