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Interactions of *Escherichia coli* O157:H7, *Salmonella typhimurium* and *Listeria monocytogenes* plants cultivated in a gnotobiotic system

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Abstract

The growth and persistence of *Escherichia coli* O157:H7, *Salmonella typhimurium* and *Listeria monocytogenes* on a diverse range of plant types over extended cultivation periods was studied. When introduced on the seed of carrot, cress, lettuce, radish, spinach and tomato all the pathogens became rapidly established shortly after germination, attaining cell densities of the order of 5.5–6.5 log cfu/g. In general, *Es. coli* O157:H7 and *L. monocytogenes* became established and persisted at significantly higher levels on seedlings (9 days post-germination) than *Salmonella*. *Es. coli* O157:H7 became internalized in cress, lettuce, radish and spinach seedlings but was not recovered within the tissues of mature plants. Internalization of *Salmonella* was also observed in lettuce and radish but not cress or spinach seedlings. In contrast, *L. monocytogenes* did not internalize within seedlings but did persist on the surface of plants throughout the cultivation period. Co-inoculation of isolates recovered from the rhizosphere of plants did not significantly affect the numbers or persistence of human pathogens. The only exception was with *Enterobacter cloacae*, which reduced *Es. coli* O157:H7 Ph1 and *L. monocytogenes* levels by ca. 1 log cfu/g on lettuce. With the bioluminescent phenotype of *Es. coli* O157:H7 Ph1, it was demonstrated that the human pathogen became established on the roots of growing plants. Scanning electron micrographs of root seedlings suggested that *Es. coli* O157:H7 Ph1 preferentially colonized the root junctions of seedlings. It is proposed that such colonization sites enhanced the persistence of *Es. coli* O157:H7 on plants and facilitated internalization within developing seedlings. The results suggest that the risk associated with internalized human pathogens in salad vegetables at harvest is low. Nevertheless, the introduction of human pathogens at an early stage of plant development could enhance their persistence in the rhizosphere. The implications of the study with regards to on-farm food safety initiatives are discussed.

Keywords: *Escherichia coli* O157:H7; *Salmonella typhimurium*; *Listeria monocytogenes*; Salad vegetables; Plant bacterial interactions

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1. Introduction

It is estimated that 14% of all foodborne illness cases reported in North America can be linked directly to minimally processed fruit and vegetables (Bean et al., 1997; Mead et al., 1999). Virulent pathogens such as *Escherichia coli* O157:H7, *Salmonella* and *Listeria monocytogenes* have all been implicated in such outbreaks of foodborne illness (Beuchat, 1996). To give examples, *Es. coli* O157:H7 has been associated with alfalfa sprouts, apple juice, cabbage, celery, cilantro, coriander, cress sprouts and lettuce (Beuchat, 2002); *Salmonella* has been recovered from tomatoes, seed sprouts, cantaloupe, apple juice, and orange juice (Beuchat, 2002); and *L. monocytogenes* has been linked with a diverse range of produce types including bean sprouts, cabbage, chicory, cucumber, eggplant, lettuce, mushroom, potatoes, radish, salad vegetable and tomato (Beuchat, 1996; National Advisory Committee on Microbiological Criteria for Food, 1999; Beuchat, 2002).

Through various studies, it has been established that the survival and interaction of human pathogens with growing plants are greater than previously thought (Warriner et al., 2003a). It has been suggested that pathogens, such as *Es. coli* O157:H7 and *Salmonella* can become internalized within growing plants and thereby are protected from any post-harvest washing treatment (Solomon et al., 2002; Watchel et al., 2002a; Cooley et al., 2003; Dong et al., 2003; Warriner et al., 2003a,b,c). To date, internalization of human pathogens in lettuce (Solomon et al., 2002; Watchel et al., 2002a), tomato plants (Guo et al., 2002), alfalfa (Gandhi et al., 2001; Dong et al., 2003), bean sprouts (Warriner et al., 2003b) and radish (Itoh et al., 1998) has been observed. However, all these studies have focused on interaction of bacteria with seedlings or sprouts exposed over short times when the potential of pathogens to become internalized is likely to be high (Warriner et al., 2003a). However, the risk posed by internalized human pathogens during extended periods of plant cultivation has yet to be established. Cooley et al. (2003) recently reported the interaction of *Es. coli* O157:H7 and *Salmonella* with growing *Arabidopsis thaliana* when introduced on the seed or seedling roots. The authors reported that pathogen levels increased significantly during seed germination and reached high numbers in the rhizosphere of seedlings. After prolonged cultivation in a soil-free system, *Es. coli* O157:H7 and *Salmonella* could be recovered from the entire plant, including seeds. Pathogen persistence was decreased when studies were performed in soil microcosms or by introduction of the rhizobacterium *Enterobacter asburiae* (Cooley et al., 2003).

In a further study, the persistence and distribution of a bioluminescent generic *Es. coli* strain introduced onto spinach seeds was investigated (Warriner et al., 2003c). In a gnotobiotic system, the *Es. coli* strain preferentially colonized the roots of emerging seedlings and became internalized. When the seedlings were cultivated hydroponically for 40 days internal *Es. coli* populations diminished, but those on the root and leaf surfaces persisted. When seedlings derived from non-inoculated seeds were cultivated in the presence of *Es. coli*, the bacterium became internalized in roots but was recovered from only the surface of leaves (Warriner et al., 2003c). Therefore, evidence to date indicates that internalization into the vascular system of plants occurs primarily in seedlings and is less prominent in mature plants. However, care has to be taken in generalizing findings as the extent of human pathogen interaction with plants is both bacterial strain and plant type specific (Dong et al., 2003). The following study investigated the interaction of virulent human pathogens with a diverse range of plant types over extended cultivation periods. The aim was to establish the microbiological risks from harvested crops that would be posed by introduction of human pathogens at the early stages of plant development.

2. Materials and methods

2.1. Bacterial strains and inocula preparation

Bacterial strains used in this study are described in Table 1. The bioluminescent or green fluorescent markers facilitate differentiation during enumeration of pathogens. However, the bioluminescent phenotype of *Es. coli* O157:H7 Ph 1 was also used to visualize the in planta distribution of the pathogen.

L. monocytogenes was cultivated aerobically overnight at 37 °C in Brain Heart Infusion broth (BHI; BD Diagnostic Systems, Sparks, MD, USA) containing 5