Manure and Contaminated Irrigation Water as Vehicles of *Escherichia coli* H157:O7 Transmission to Fresh Produce

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**INTRODUCTION**

- The majority of *Escherichia coli* (E. coli) found in the environment are considered harmless but data show that in the US some 70,000 people are infected by *E. coli* O157:H7 every year [5], resulting in an annual cost of $405 million for health related expenses and lost productivity.
- The consumption of contaminated water is considered the most common route of infection, but outbreaks associated with *E. coli* 0157:H7 have also been reported from the consumption of contaminated raw vegetables and fruits [2, 3, 6].
- How *E. coli* O157:H7 contaminate produce is not clear in all cases, but transmission to fresh produce when the plants are grown in soils receiving manure application (4) or irrigated with contaminated water [1, 7] is suggested as possible pathways. The role that applications of animal waste and contaminated irrigation water play as sources for bacterial transfer is not clear.
- The objective of this study was to understand the factors controlling the transmission of *E. coli* from soil and irrigation water to radish and lettuce by taking advantage of *E. coli* strains modified with *gfp* and *lux* insertions in order to understand the relationship.

**MATERIALS & METHODS**

1. **Bacteria Used**

- *E. coli* O157:H7 strain marked with the *lux* and *gfp* genes was employed to track and quantify the activity and distribution of bacteria in agar and soil systems.

2. **Growing Plants**

   **Agar System**

   - Radish and lettuce plants were grown from *lux-E. coli* H157:O7 contaminated seeds, a scenario that is possible when seeds are sown in field irrigated with contaminated water or if the field has a history of manure application.
   - The plants were grown in test tubes (Figure 3A) containing Hoagland’s nutrient solution solidified with agar (0.8 %) for a short-term study.
   - The agar system was convenient to easily track *E. coli* transmission throughout the plant during growth.

   **Soil System**

   - *E. coli* was introduced into soil in a number of different ways. (Figure 4)
   - No *E. coli*
   - Control - C
   - Seeded before treatment
   - Direct mixing with soil - ES
   - Manure - EM
   - Irrigation - EI
   - Manure & Irrigation - EMI
   - Seeded with lettuce or radish after treatment

3. **RESULTS AND DISCUSSION**

- *E. coli* growth and transmission in Agar system

   - Due to the energy dependency of bioluminescence (Figure 2), the light intensity can be correlated to the metabolic activity of the bacteria.

   - *Gfp* marked *E. coli*, on the other hand, fluoresces under UV light and could be used to track the bacteria in environmental matrices.

   - *E. coli* strain 2+, marked with multiple antibiotics, was also used to trace its transmission in soil where plasmid based *gfp* trait was unstable.

   - The control plants were not visible due to the absence of *lux-E. coli*.

- The distribution of *lux-E. coli* on plants was captured with a CCD (charge coupled Device) camera.

- *E. coli* (10^6/10^7 cfu g^-1/mL^-1) applied to soil.

- Plants kept in growth chamber (Figure 3B) at 19-25°C & 16/8 light/dark cycle.

- *E. coli* extraction - shaking soils in Na-phosphate buffer; stomaching for plant samples.

- *E. coli* number - Plate count and TaqMan Real-time PCR targeting the *eae* gene.
All the treatments resulted in significant number of gfp-E. coli on radish root and leaves, with EM and EMI resulting in higher # on roots and bulk soil than the others. Radish grown in EI treated soils had higher E. coli # on leaves than ES or EM.

Gfp-E. coli was not detected in bulk soil and leaves after 14 days of growth but was present in rhizosphere until day 28. The disappearance of gfp-E. coli afterwards might not indicate total absence/death but the loss of the gfp phenotype that needs selected pressure for maintenance (Figure 7). E. coli strain 2+ was marked with multiple antibiotics and used to avoid this problem.

Real-time PCR assay was successfully used to quantify E. coli H157:O7 in the samples but was limited in use because of its high detection limit (10^4/10^5 cfu g^-1) in soils.

The lettuce study (Figure 8) showed that E. coli can be transmitted to leaves if clean soil is irrigated with contaminated water after several days of lettuce growth. Unlike the gfp-marked E. coli, the 2+ strain was present at high number in the rhizosphere and bulk soil late in the growth stage but was not detected on leaves after day 41.

The agar system was used because of the convenience to track the transfer of E. coli throughout the plant. Glowing roots and leaves of radish and lettuce (Figures 5 & 6) indicated the presence of lac-E. coli on these surfaces, showing its transmission from the seeds to the plant parts.

The light intensity (due to the lac-E. coli) from radish root and leaves increased a few days after germination (Figure 5), indicating increased E. coli metabolic activity. Increased E. coli number on seedlings versus the original number on seeds (Figure 7) indicated the correlation of light intensity with the increase in number.

The combination of increased light intensity and number indicated the availability of carbon and other nutrients for E. coli use and growth, especially during the early stages of germination (e.g., radish sprouts).

Soil system: E. coli growth and transmission

Table 1: Concentration of gfp-E. coli (CFU g^-1) in soil, leaves and rhizosphere of radish plants grown in soil that received one time E. coli application at 10^8 CFU ml^-1/g via ES (mixing with soil), EI (irrigation), EM (manure) and EMI (manure and irrigation). *ND = not detectable.

<table>
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<th>Plant part</th>
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SUMMARY & CONCLUSIONS

- Radish and lettuce plants grown in nutrient beefed agar from contaminated seeds showed significant amount of lac-E. coli O157:H7 on their root and leaves, as captured by CCD camera and plates counts, indicating the survival and transmission of E. coli on growing plants.

- When E. coli was introduced into soil via irrigation, manure or direct mixing, it established itself on the root and leaves of radish and lettuce grown in the soil. Introduction via manure resulted in the highest E. coli # in the rhizosphere while irrigation resulted in higher E. coli # on leaves than manure. E. coli was not, however, detected on radish or lettuce leaves towards the end of the growing period in all the treatments.

- The study highlighted the ability of E. coli to establish itself on growing vegetables especially at early growth stage with implications for sprout safety while its persistence in rhizosphere might facilitate internalization that could render traditional sanitary measures ineffective.

ACKNOWLEDGMENTS

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REFERENCES