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TRANSMISSION OF *E. COLI* FROM CONTAMINATED IRRIGATION WATER AND SOIL TO PLANT TISSUE

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Abstract

The number of documented outbreaks of human infections associated with the consumption of raw fruits and vegetables has increased in recent years. Among the greatest concerns with human pathogens on fresh vegetables are enteric pathogens. *Escherichia coli* and *E. coli* O157:H7 infections have been associated with lettuce, sprouts and carrots.

The goal of this research was based on transmission *E. coli* from contaminated irrigation water and soil to plant tissue of lettuce and detection of *E. coli* inside plant tissue.

Bacterial strain used in this experiment was *E. coli* K-12 W3110 and model plant was lettuce. The soil for growing plants was irrigated with contaminated water. The detection of *E. coli* in plant tissue was done by PCR method. The visualization of bacteria inside plant tissue was done by hand-cutting parts of plants and seeing under confocal laser scanning microscope.

Results showed that treatments with contaminated water increased the number of *E. coli* inside root and leaves. PCR-based assay confirmed presence of *E. coli* inside lettuce root and leaves. Also, by confocal laser scanning microscopy, it was seen *E. coli* inside roots and leaves.

It suggests that pathogenic bacteria could enter inside leaf through stomata as well as through vascular root system if it is used contamination irrigation water.

Key words: *E. coli, lettuce, contaminated irrigation water.*

1. Introduction

The number of documented outbreaks of human infections associated with the consumption of raw fruits and vegetables has increased in recent years.

More recently, salmonellosis has been linked to tomatoes, seed sprouts, and cantaloupe (Beuchat [1]). *Escherichia coli* O157:H7 infection has been associated with lettuce, sprouts and enterotoxigenic *E. coli* has been linked to carrots. Documented associations of shigellosis with lettuce and parsley have been made. *E. coli* O157:H7 has been isolated from: alfalfa sprouts, cabbage, celery, coriander, cress sprouts, lettuce and *Salmonella* has been isolated from: alfalfa sprouts, artichokes, beet leaves, celery, cabbage, cantaloupe, cauliflower, eggplant, green onions, lettuce, parsley, spinach, tomato (Buck *et al.* [2]).

Identifying primary inoculum sources for contamination of fresh produce can be tremendously difficult. For example, only two of 27 outbreak investigations described in the NACMCF (National Advisory Committee on Microbiological Criteria for Foods [3]) report on fresh produce clearly identified a point of contamination.

Bacterial pathogens may contaminate vegetables at any point throughout the production system. Potential pre-harvest sources of contamination include soil, faces, irrigation water (Kljujev [4]), water used to apply fungicides and insecticides, dust, insects, inadequately composted manure, wild and domestic animals, and human handling (Beuchat [5]). Transmission of *E. coli* O157:H7 from manure-contaminated soil and irrigation water to lettuce plants and its migration throughout the plant were recently reported (Solomon *et al*. [6]). Evidence of an association of salmonellae with stems and leaves of tomato plants grown hydroponically in inoculated solution has been presented (Guo *et al*. [7]).

The goal of this research was better understanding the contamination of food crops by human pathogens and devising better management options to limit human

infections. The investigation is based on: transmission *E. coli* K-12 W3110 from contaminated irrigation water and soil to plant tissue of lettuce (*Lactuca sativa*), detection of *E. coli* K-12 W3110 inside plant tissue and possibility their transport through plant vascular system.

2. Materials and Methods

Bacterial strain used in this experiment was *E. coli* K-12 W3110. Model plant was lettuce (*Lactuca sativa*). The soil for growing plants was irrigated with contaminated water, and the concentration of bacteria was approximately 10^9 cells/mL ($OD_{650} \approx 1$). Plants were grown in triplicate and with non-inoculated control plants (irrigated with sterile water). Seeds were sterilized in 10% ethanol and than washed in sterilized dH_2O, before planting. The investigated plants grew two weeks.

After two weeks, plants were harvested. To investigate the presence of bacteria inside plant tissues, surfaces of plants were sterilized in the 70% ethanol to eliminate any surface colonization. After that, the 1 g of root and 1 g of leaves were cut and macerated in sterile $MgCl_2$ solution. Then, dilutions for root and leaf extract were prepared and plated on MacConkey Agar (Oxoid, UK) in triplicate. The number of pink colonies, estimated as *E. coli*, was counted and bacterial numbers estimated per g plant tissue. The same was done for soil samples.

For checking and identification of *E. coli* K-12, the API 20E kit was employed and results analyzed using API-WEB (Biomerieux, France). The detection of *E. coli* K-12 W3110 inside and on the surface of plant tissue (root and leaves) was done by PCR method using appropriate primers for the bacterial strain employed. The primer was feoA BW25113, for: 5' – GAA ACC TTA ATT AAA CAT TAG CCA GTC CGG – 3' and rev: 5' – GCC ACT CAA AAT GTA GTG ACA GGC GAT T – 3'. The Marker was HyperLadder™ I (Bioline Quantitative DNA Markers). The visualization of bacteria inside plant tissue was achieved by hand-cutting parts of plants followed by observation under confocal laser scanning microscope. The Confocal Laser Scanning Microscope was a Leica inverted DM IRE2 and the image capture software was TCS SP2 AOBS. Plant samples (root and leaves) were stained with 0.1 mg/mL PI for 10 minutes. The objective was x 40 oil immersion 1.25 NA (numerical aperture) Green Helium Neon laser: Excitation 543 nm, Emission 593-726nm.

3. Results and Discussion

Microbiological results indicated a big potential risk if contaminated water is used for irrigation of lettuce plants. Results showed that treatments with contaminated water resulted in significant levels of *E. coli* inside root and leaves, whereas un-inoculated controls were free of detectable *E. coli* contamination (Fig. 1). The very high number was found on the surface of roots (4.1 x 10^5 CFU) and the lowest was inside leaves (5.0 x 10^2 CFU). Quite high numbers of *E. coli* were found inside lettuce root, 3.0 x 10^3 CFU. The highest number of *E.coli* was found in the soil, near the root (7.3 x 10^5 CFU). *E. coli* K-12 was not detected on the surface of lettuce leaves, or inside and outside root and leaves in control plants.

Isolated strains of *E. coli* fermented lactose and gave typical pink colonies on the MacConkey Agar. Colonies derived from bacteria already associated with the root of control plants were quite different and so were clearly distinguishable. Biochemical tests showed that isolated strains of bacteria belong to *E. coli* K-12 which was used for irrigation water contamination.

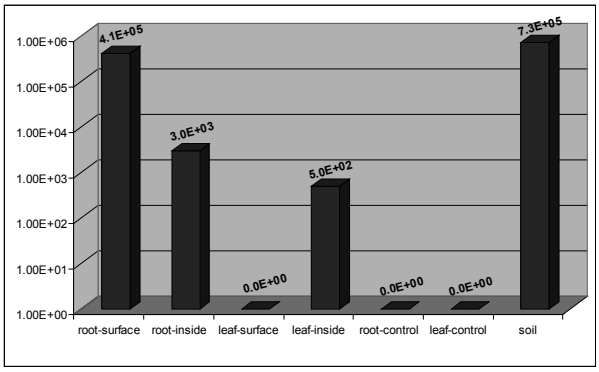


Figure 1. Number of E. coli K-12 (CFU) inside and on the surface of root and leaves of lettuce and in the soil (per 1 g)

PCR-based assay also confirmed that the characteristic pink colonies (on McConkey Agar) isolated from surface and inside of root, as well as inside of leaves, are *E. coli* K-12 (Fig. 2). The, non pink colonies isolated from root and leaves (surface and inside) are not *E. coli* K-12 because there were did not give the PCR product which is characteristic for *E. coli* K-12 (Fig. 2 – line I and J). The PCR product in the line H (positive control *E. coli* K-12 W3110) is same as in the lines A, B, C, D, F and G and it is exactly 600 bp.

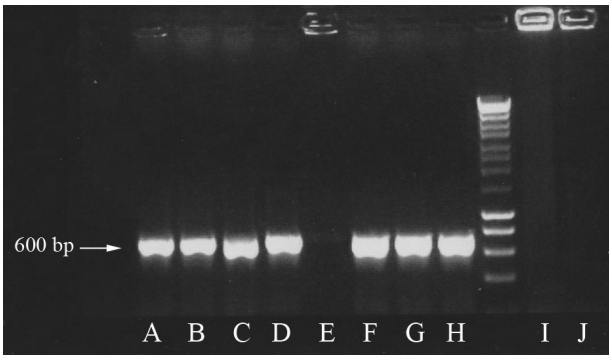


Figure 2. Detection of PCR-based assay of E. coli K-12 isolated from root and leaves of lettuce (lines A and B – *E.*

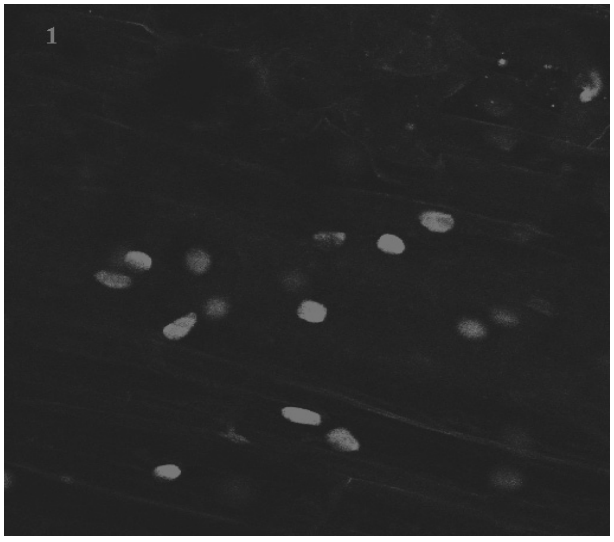
***coli* K-12 strains isolated form surface of root; lines C and D - *E. coli* K-12 strains isolated form inside of root; lines F and G - *E. coli* K-12 strains isolated form inside of leaves; line H – *E. coli* K-12 control strain (positive control) ; line I – non pink colony isolated from root; line J - non pink colony isolated from leaves)**

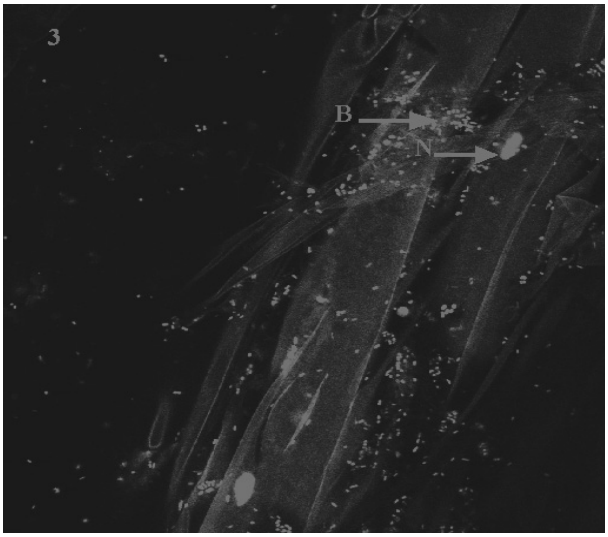
Microscopy confirmed the presence of *E. coli* inside root and leaves of lettuce (Figure 3). *E. coli* was not found inside root of control plants but nucleus of root cells were clearly seen (Figure 3; 1). On picture 3, plant vascular tubes, bacteria and root cell nucleus inside them in the same layer can be seen. This suggests that bacteria could be transported though the vascular system of the plant to edible parts of plant.

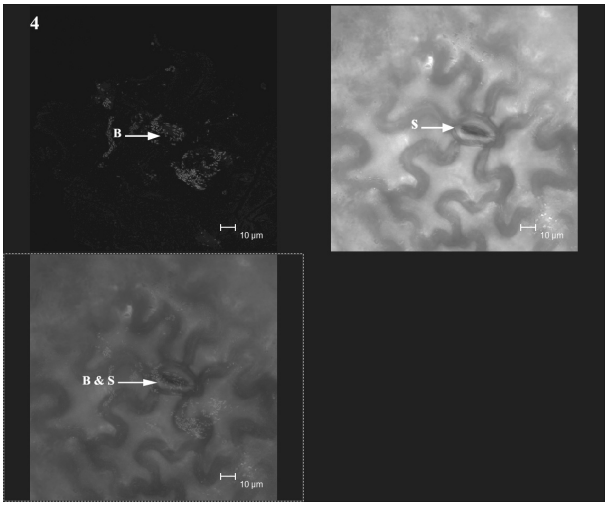
Also, by confocal laser scanning microscopy, *E. coli* was seen inside leaves and microcolonies of *E. coli* were detected below the surface of leaves. *E. coli* cells were concentrated near stomata (Figures 4 and 6). This suggests that pathogenic bacteria could enter the inside of a leaf through stomata if present in irrigation water.

In the root, bacteria started to appear inside the root tissue at a depth of 10 µm deep but, far from root surface, they could not be seen at a depth of more than 22 µm. The highest concentration of bacteria was found in the layers at a depth of 19-20 µm. Also, this depth gave the best visibility for the presence of bacteria inside the plant vascular system.

In the leaves, bacteria started to appear in at a depth of 6 µm, and far from the leaf surface, bacteria were still visible at a depth of 16 µm. The best visibility of bacteria inside leaves was in the layer 11 µm deep. On the surface of leaves, confocal microscopic observation did not show the presence of bacteria.







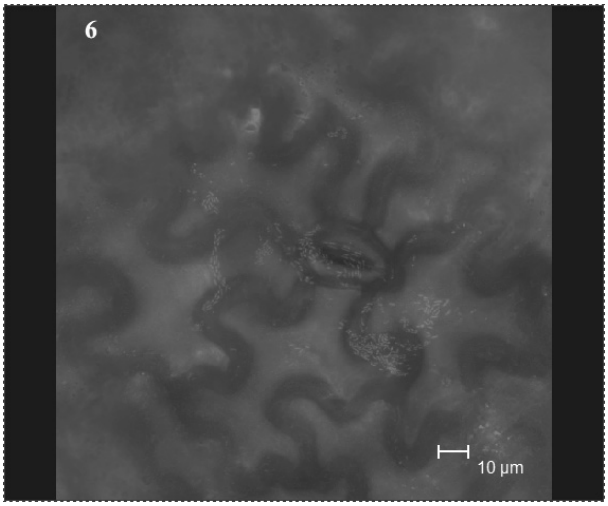


Figure 3. Micrographs of lettuce roots and leaves, 1. Root of control lettuce plant irrigated with sterile water; picture 3 (the layer is 20 µm deep) – root of plants irrigated with contaminated water; 4 and 6 (the layer is 11 µm deep). Leaves of plant irrigated with contaminated water; B – bacteria cells, N – nucleus of plant cells, S – stomata

The results show that *E. coli* can enter the roots of young lettuce plants and can be transported upward to locations within the leaves of the plant (Figure 3). Direct contact between the leaves and contaminated irrigation water is not required for the bacteria to become integrated into lettuce leaves. This means that irrigation with *E. coli* contaminated water may result in contamination of the crop in the field. According to Solomon *et al*. [6], application of manure contaminated with *E. coli* O157:H7 to the production field, may contaminate crops. The source of contamination could be manure as well as contaminated water (Kljujev [4], Dulic *et al*. [8]). Some studies show that pathogenic bacteria, especially *E. coli*, can survive very long periods in water (Chalmers *et al*. [9]). Also, bacterial human pathogens can survive for a long period in animal manures and soils as well as on growing plant tissue (Gagliardi [10]).

The levels of *E. coli* K-12 used in this study are much higher than what could be found on an agricultural field. High numbers of bacteria were used to enable easy detection of bacteria in the investigated parts of the lettuce plant. However, under natural conditions, even a low level of pathogenic bacteria in the environment could present a significant human health risk.

Our research suggests that surface sanitizing of lettuce is not an effective method to eliminate all *E. coli* cells, because bacterial cells exist inside of lettuce plants (Beuchat [11]). The inaccessibility of a many endophytic bacteria, as a consequence of their subsurface location, is perhaps the reason for the lack of effectiveness of surface-sanitizing treatments (Solomon *et al*. [6]).

The application of contaminated water for irrigation which may result in *E. coli* K-12 and other pathogen bacteria becoming associated with lettuce, or for other crops, have not been sufficiently explored.

4. Conclusions

We found *E. coli* K-12 not only on outer surfaces but also in inner tissues of root and near stomata when sprouts are raised from *E. coli* bacteria-contaminated seeds. The treatment of the outer surfaces of cotyledons with 70% ethanol does not kill the bacteria. We also found viable bacteria, after surface sterilization with 70% ethanol, around the vessels, close to stomata of leaves and in the vascular system of root.

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