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Exposure of *Escherichia coli* O157:H7 to soil, manure, or water influences its survival on plants and initiation of plant defense response

Seungwook Seo, Karl R. Matthews*

Department of Food Science, Rutgers University, New Brunswick, NJ 08901, United States

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ABSTRACT

This study evaluated whether growth medium or exposure conditions influence the production of capsular polysaccharides (CPS) by *Escherichia coli* O157:H7, and whether changes in CPS impact plant defense responses, consequently affecting survival on plants. *E. coli* O157:H7 grown in Luria–Bertani (LB) broth supplemented with manure extracts showed an approximately 58% increase in CPS production compared to cells grown in LB medium alone. Levels of CPS were significantly higher for *E. coli* O157:H7 cells exposed to soil or manure extracts as compared to the non-exposed LB cultured control. *Arabidopsis thaliana* plants expressing β-glucuronidase (*GUS*) under the control of the β-1,3-glucanase (*BGL2*) promoter were used to investigate whether *E. coli* O157:H7 induces defense-related gene expression. Plants inoculated with *E. coli* O157:H7 grown in LB containing manure extracts or cells exposed to manure extracts exhibited 3-fold and 2-fold lower GUS activity, respectively, suggesting a limited plant defense response compared to plants inoculated with cells grown in LB. On day 5 post inoculation the population of *E. coli* O157:H7 grown in LB supplemented with manure on plants was significantly greater than the population of *E. coli* O157:H7 grown in LB medium alone. *E. coli* O157:H7 cells exposed to soil or manure exhibited greater survival on plants compared to LB-grown *E. coli* O157:H7. The results of this study underscore the need to consider medium composition and cultural conditions when conducting crop challenge studies.

1. Introduction

During the past decade, fresh produce, such as alfalfa sprouts, lettuce, spinach, and parsley have been linked to outbreaks of foodborne illness. The increase in foodborne illness associated with consumption of fresh fruits and vegetables has been attributed to increased consumption of fresh produce as well as changes in agronomic, harvesting, processing, and distribution patterns (Franz et al., 2011). Crops in the field can be contaminated through exposure to manure, soil, irrigation water, or animal feces harboring pathogenic microorganisms (Sharma et al., 2009). Previous studies have shown that human pathogens can survive and persist for a long period of time in the field environment such as soil and animal feces (Islam et al., 2004; Semenov et al., 2008). Healthy cattle are known to be a major reservoir of human pathogens and shedding of pathogens can result in contamination of soil and water. The survival of enteric pathogens outside the host may be greatly affected by environmental conditions where bacteria encounter temperature fluctuation, limited nutrients, and UV radiation (Brandl, 2006).

Bacterial cell surface structures including curli, flagella, and extracellular polysaccharides play an important role in the interactions between enteric pathogens and abiotic and biological surfaces. Those structures were reported to mediate bacterial attachment, colonization, or biofilm formation on plant tissue (Barak et al., 2005, 2007; Xicohtencatl-Cortes et al., 2009). The expression of bacterial surface components has been shown to be influenced by culture conditions and nutrient composition of the growth medium (Bonet et al., 1993; Hassan and Frank, 2004; Li et al., 1993; Olsén et al., 1993). The term extracellular polysaccharides refer to capsular polysaccharides (CPS) and exopolysaccharides (EPS) or slime. EPS are loosely bound to the cell surface, and easily sloughed from the cell surface, while CPSs are tightly associated with the cell surface (Roberts, 1996). CPSs protect bacterial cells from adverse environments associated with desiccation, osmotic or oxidative stresses, confer resistance to antimicrobial

* Corresponding author. Tel.: +1 848 932 5404.
E-mail address: matthews@aesop.rutgers.edu (K.R. Matthews).

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compounds, and aid in the evasion of the host immune response (Campos et al., 2004; Roberts, 1996).

Research on the interaction of foodborne pathogens with crops grown in the field or under laboratory conditions has increased dramatically as researchers seek to develop new strategies to improve the safety of fresh fruits and vegetables. The growth conditions that bacteria are subjected to prior to inoculation of crops used in those studies may have a dramatic influence on the outcome of the research. Cell surface moieties associated with a bacterium cultured in a laboratory medium (e.g., LB broth) may differ from cell surface moieties expressed by the same bacterium when found in harsh environments. Differences in expression of extracellular components may significantly impact how the bacterium interacts with plants. The effects of growth medium on cell surface properties, such as EPS, cell size, hydrophobicity and surface charge have been reported in the scientific literature (Hassan and Frank, 2004; Marcus et al., 2012).

Research on the plant defense response to human pathogens is limited compared to that on plant pathogens. Recent studies have shown that *Salmonella* and *Escherichia coli* O157:H7 colonization induced SA (salicylic acid)- or JA (jasmonic acid) -regulated plant defense responses (Melotto et al., 2006; Schikora et al., 2008; Seo and Matthews, 2012; Shirron and Yaron, 2011). In this study, we investigated the effects of growth medium (Luria–Bertani [LB] broth, and LB supplemented with soil or manure extracts) or exposure to carrier vehicle environments (soil or manure extracts) on CPS production. CPS was a logical cell surface moiety to use as a marker demonstrating the influence of growth conditions on *E. coli* O157:H7 since changes can be monitored qualitatively and quantitatively and it is associated with survival and virulence (Roberts, 1996). Moreover, the influence of *E. coli* O157:H7 CPS on plant defense responses and the impact on bacterial survival on a plant have not been investigated previously. The objectives of this study were to investigate whether growth medium or exposure to the variable environments encountered in carrier vehicles influence the production of bacterial CPS, their possible role as elicitors of plant defense responses and consequent effects on the survival of *E. coli* O157:H7 on plants.

2. Methods

2.1. Bacterial strain and inoculum preparation

E. coli O157:H7 (ATCC 43895) transformed with plasmid pGFP was grown on tryptic soy agar (TSA: Difco, Becton Dickinson, Sparks, MD) supplemented with ampicillin (Sigma–Aldrich, St. Louis MO), at 100 μg/ml at 37 °C. Stock cultures were maintained in Tryptic soy broth (TSB: Difco, Becton Dickinson, Sparks, MD) containing 30% glycerol at −70° C. Fresh cow manure and soil were obtained from the Rutgers dairy farm and the Rutgers greenhouse (New Brunswick, NJ), respectively. Upon receipt manure and soil samples were suspended in sterile distilled water (1:10, w/v), and filtered with cheese cloth to remove large particles. The resulting slurry was spun at 3500 × *g* for 20 min and the decanted solutions were filtered through a 0.2 micron sterile filter (Nalgene). Filtered soil and manure extracts were stored at −20 °C until needed. LB media (Difco) supplemented with soil extract (LB + Soil) or manure extract (LB + Manure) were prepared by mixing a 2× concentrated LB and the soil or manure extracts at a ratio of 1:1 (v/v).

Exposure of *E. coli* O157:H7 to soil or manure extracts was conducted as follows. An overnight culture grown in LB broth was spun at 5000 × *g* for 10 min at 4 °C and the resulting pellet was resuspended in sterile distilled water. The suspension was spun under the same conditions and the cells were resuspended in soil or manure extracts. The bacterial suspensions in soil extract or manure extract were held at room temperature (22 ± 2 °C) for 5 days to simulate passage through each environment prior to interaction of the cells with plants.

2.2. Plants and growth conditions

Seed of *Arabidopsis thaliana* ecotype Columbia (Col-0) wild-type (CS 70000) was obtained from the Arabidopsis Biological Resource Center, The Ohio State University. Seeds of Col-0 transgenic line (*BGL2*::*GUS*) were kindly provided by Dr. Xinnian Dong (Duke University, NC, USA). *BGL2-GUS* transgenic plants contain a β-glucuronidase reporter gene (*GUS*) driven by the β-1,3-glucanase (*BGL2*) promoter (Cao et al., 1994). *Arabidopsis* plants were grown in Metromix 360 soil in a climate-controlled greenhouse (22 °C ± 2 °C, relative humidity 70% ± 5%) under natural light supplemented with artificial light to achieve 16 h per day of light.

2.3. Exposure of E. coli O157:H7 to growing plants

Bacterial cells (prepared as described above: grown overnight at 37 °C in LB, LB + Soil, and LB + Manure, or exposed to soil or manure extracts) were harvested by centrifugation and resuspended in sterile distilled water (SDW) to achieve approximately 10^8 CFU/ml. Serial (1:10) dilutions prepared in sterile phosphate buffered saline were spread in duplicate on TSA plates which were incubated at 37 °C for 18 h to determine number of viable cells in each suspension.

Plant inoculation: Five week-old wild-type plants were dipped in a bacterial suspension for 30 s and then placed in a growth room under a transparent plastic dome for 1 day to maintain high humidity. Control plants were treated with sterile distilled water and grown as described. At 0, 3, and 5 days post-challenge three plants from each treatment group were harvested. Weighed plant samples were placed in a sterile stomacher bag with 0.1% peptone (w/v), macerated by hand, and then homogenized for 3 min using a stomacher. Appropriate dilutions of the homogenates were spread in duplicate on TSA supplemented with ampicillin (100 μg/ml). Following incubation at 37 °C for 18 h the colonies were counted and populations were reported as log CFU per g fresh weight of plants. The experiment was conducted twice. For the GUS activity experiment, 4 week-old *BGL2-GUS* transgenic plants were dipped in a bacterial suspension as described above. For the negative and positive controls, plants were dipped in sterile distilled water or 1 mM salicylic acid (SA) for 30 s, respectively. At day 5 post inoculation, plants were harvested and GUS assays were conducted.

2.4. Quantitative analysis of GUS activity

Whole plants were homogenized using a pre-chilled micro pestle in 200 μL of GUS extraction buffer (50 mM Na_2HPO_4, pH 7.0, 10 mM EDTA, 0.1% Triton X-100, 0.1% SDS, 10 mM β–mercaptoethanol) and were spun for 20 min at 16,000 × g at 4 °C (Jefferson et al., 1987). Protein concentration and enzyme activity were measured in the supernatant. Protein concentrations were determined by the Bradford method, and GUS reactions were carried out by adding 20 μL of extracts to 980 μL of GUS extraction buffer containing 1 mM MUG (4-methylumbelliferyl-β-D-glucuronide) at 37 °C. After 0, 10, 20, or 40 min incubation, 200 μL from each reaction was added to a test tube containing 800 μL 0.2 M Na_2CO_3 to stop the reactions. Fluorescence was measured using a Perkin Elmer LS-50B luminescence spectrometer (Perkin Elmer, Branford, CT) with excitation at 365 nm, emission at 455 nm, and slit width of 5 nm. GUS activity was quantified using freshly prepared 4-MU (4-