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Using the agricultural environment to select better surrogates for foodborne pathogens associated with fresh produce

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ABSTRACT

Despite continuing efforts to reduce foodborne pathogen contamination of fresh produce, significant outbreaks continue to occur. Identification of appropriate surrogates for foodborne pathogens facilitates relevant research to identify reservoirs and amplifiers of these contaminants in production and processing environments. Therefore, the objective of this study was to identify environmental *Escherichia coli* isolates from manures (poultry, swine and dairy) and surface water sources with properties similar to those of the produce associated foodborne pathogens *E. coli* O157:H7 and *Salmonella enterica* serotype Typhimurium. The most similar environmental *E. coli* isolates were from poultry ($n = 3$) and surface water ($n = 1$) sources. The best environmental *E. coli* surrogates had cell surface characteristics (zeta potential, hydrophobicity and exopolysaccharide composition) that were similar (i.e., within 15%) to those of *S. Typhimurium* and/or formed biofilms more often when grown in low nutrient media prepared from lettuce lysates (24%) than when grown on high nutrient broth (7%). The rate of attachment of environmental isolates to lettuce leaves was also similar to that of *S. Typhimurium*. In contrast, *E. coli* O157:H7, a commonly used *E. coli* quality control strain and swine isolates behaved similarly; all were in the lowest 10% of isolates for biofilm formation and leaf attachment. These data suggest that the environment may provide a valuable resource for selection of surrogates for foodborne pathogens.

1. Introduction

Contamination of food and water by pathogens continues to be a significant public health concern in the United States (U.S.). It is estimated that 48 million foodborne illnesses occur each year and only 9.4 million of those are caused by identified pathogens (Scallan et al., 2011). A substantial percentage of illnesses (around 46%) are thought to be associated with fresh produce (Painter et al., 2013) and leafy vegetables have been categorized as a food safety priority by the World Health Organization (WHO; FAO/WHO, 2008). Although the incidence of some important foodborne pathogens, including *Salmonella* sp. and *Escherichia coli* O157:H7, has decreased in recent years, significant outbreaks continue to occur (Crim et al., 2015; Goodburn and Wallace, 2013; Olaimat and Holley, 2012). This is despite implementation of recommended good agricultural practices (GAPs) targeted to fresh fruits and vegetables (Goodburn and Wallace, 2013; Painter et al., 2013). Although most of the recommended mitigation strategies have focused on post-harvest processing, environmental monitoring programs for pre-harvest safety are increasingly important. Studies have shown the importance of the environment (irrigation water, soil, wildlife) as the source for contaminants (Gelting et al., 2015; Jung et al., 2014; Olaimat and Holley, 2012; Van Boxstael et al., 2013) and the FDA Food Safety and Modernization Act (FSMA) has prioritized prevention by strengthening safety on the pre-harvest side of production.

National, international and industry groups have called for identification of improved surrogates that persist in ways that better mimic the behavior of important foodborne pathogens (Cabrera-Diaz et al., 2009; Deng et al., 2014; Kim and Harrison, 2009; Lemarchand and Lebaron, 2003; Sinclair et al., 2012; Ulbrich et al., 2015; Van Boxstael et al., 2013). Nonpathogenic bacteria like *E. coli* are often used by industry and regulatory groups as proxies, surrogates and/or indicators of human pathogens (Harwood et al., 2005; Jenkins et al., 2011; Keeling et al., 2009; Sinclair et al., 2012; Ulbrich et al., 2015). In production and processing environments, surrogates are used in studies to identify risk and to develop improved management practices to reduce

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pathogen contamination. Properly quantifying and mimicking the behavior of pathogens in these environmental systems is complicated and many groups have questioned the validity of using generic organisms as indices for evaluating the microbiological quality of water, soil and produce (Field and Samadpour, 2007; Griffith et al., 2009; Harwood et al., 2005). However, years of precedence, including long-term use in regulated monitoring, as well as the availability of specific, sensitive, user and budget friendly methods make *E. coli* an ideal surrogate for pathogens. The need lies in uncovering strains and properties of this or similar organisms that make them better representatives of the pathogens (Deng et al., 2014; Griffith et al., 2009; Keeling et al., 2009; Marshall et al., 2005). Having access to a representative selection of well-characterized pathogen surrogates would provide the produce industry with a viable option for evaluating and validating intervention strategies while assuring safety and simplicity of use for research and quality control purposes.

Surrogates are dissimilar to fecal indicator organisms, in that they must behave like the pathogen in a specific environment. In contrast, an indicator simply signifies the potential risk for bacterial contamination in the source environment (Sinclair et al., 2012). In general, generic *E. coli* strains such as the American Type Culture Collection (ATCC) strain *E. coli* 25922 have been used as surrogates for pathogenic *E. coli* strains such as O157:H7 and for other research, benchmarking or standard laboratory and industrial testing as recommended by regulatory groups (AOAC, 2002; Feng et al., 2011; ISO, 2014; Kim and Harrison, 2009). These strains are often adapted to laboratory conditions and lack factors that permit persistence under harsher environmental conditions.

Environmental *E. coli* isolates survive extended periods of time in the secondary habitats (soil, manure, or water) where temperatures, moisture levels, UV radiation from the sun, salinity, and other environmental conditions can create a stressful environment (Holley et al., 2006; Ishii and Sadowsky, 2008; Islam et al., 2004; Olaimat and Holley, 2012). Strain-level differences in physical, chemical and biological properties of environmental isolates of *E. coli* is extensive and likely serves to improve chances for survival in the host environment and increase fitness in secondary habitats like soil and water sources (Bolster et al., 2010; Cook et al., 2011; Dixit et al., 2004; Foppen et al., 2010; Méric et al., 2013; Son et al., 2009; Yang et al., 2004). Therefore, selection of more appropriate surrogates may be made possible by using isolates from the same niche as the enteric pathogens (i.e., gastrointestinal bacteria) that are capable of survival in secondary habitats (i.e. following fecal deposition into the environment). This approach has already been applied in the meat processing industry where biosafety level 1 (BSL-1) isolates of *E. coli*, obtained from beef hides are being used as surrogates for *E. coli* O157:H7 (Cabrera-Diaz et al., 2009; Keeling et al., 2009; Marshall et al., 2005). To our knowledge this approach has not yet been applied for identifying surrogates for foodborne pathogens associated with fresh produce. Therefore, the goal of this research was to identify *E. coli* strains from manure and surface water sources which possess genotypic and phenotypic properties similar to those of foodborne pathogens found in association with fresh produce (Bolster et al., 2009; Brandl, 2006; Cook et al., 2011; Olaimat and Holley, 2012; Yaron and Romling, 2014).

2. Materials and methods

2.1. E. coli cultures and bacterial growth conditions

This work builds on previous research in which environmental *E. coli* isolates (n = 1346) from poultry, swine, dairy and surface water sources were characterized (Cook et al., 2011). That research was used as the basis for selection of a sub-set of environmental *E. coli* isolates (n = 63) for use in this study. Selected isolates equally represented poultry, swine and dairy manure and surface water sources, included isolates with genes important for survival in secondary habitats and represented each of the *E. coli* phylogroups. These 63 *E. coli* isolates

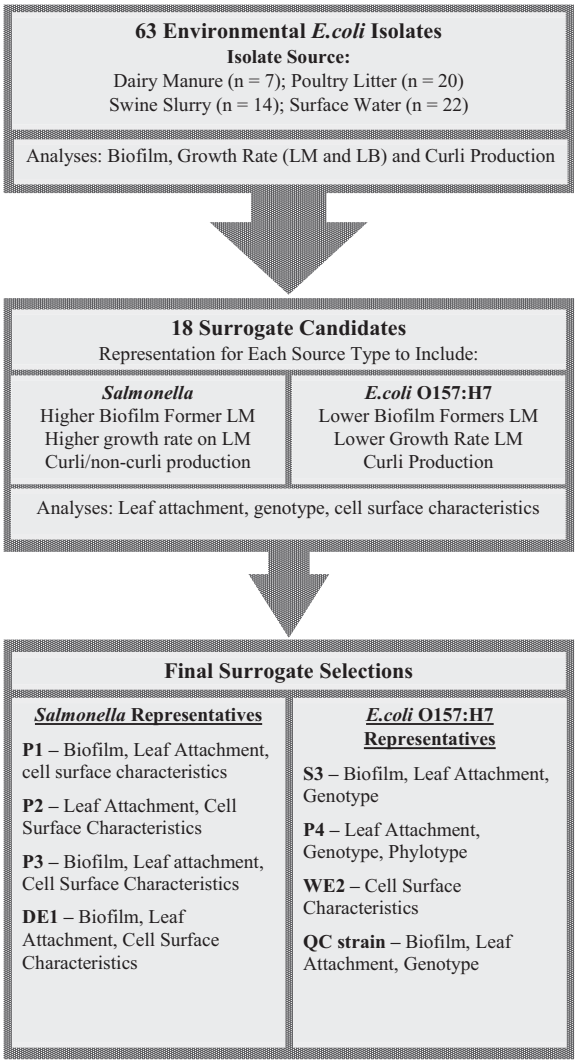

Fig. 1. Flow diagram illustrating procedures used to select environmental *E. coli* surrogates to represent produce associated foodborne pathogens (*S. Typhimurium* (*Salmonella*) and *E. coli* O157:H7). The initial *E. coli* isolates (n = 63) were a subset of 1346 isolates from dairy manure (D), poultry litter (P), swine slurry (S) or surface waters (DE or WE) and also included the common quality control (QC) strain *E. coli* ATCC 25922. Biofilm formation, growth rates and curli production were used as selection criteria to narrow the surrogate pool to 18 isolates (surrogate candidates). Candidates were assessed for lettuce leaf attachment, genotype, and cell surface characteristics. Final *E. coli* surrogate selections included four poultry litter, two surface water and one swine isolate as well as the QC strain. LM = lettuce lysates with minimal salts media; LB = Luria-Bertani broth. See Table 2 for genotype and phylogroup data and Fig. 4 for lettuce attachment data.

were then characterized to identify surrogates with genotypic and phenotypic properties similar to those reported in the literature to be associated with fitness of human pathogens in environmental sources and on produce (Barak et al., 2005; Brandl, 2006; Cook et al., 2011; Nagy et al., 2015; Tan et al., 2016; Wang et al., 2016; Yang et al., 2004; Yaron and Romling, 2014). Biofilm formation, leaf adhesion, growth rates and genes associated with adhesion, biofilm formation and fitness were used as the primary selection criteria for isolates to be used for subsequent studies. Two enteric pathogens, *E. coli* O157:H7 (ATCC 43888) and *S.* Typhimurium (ATCC 13311), and a commonly used quality control strain (*E. coli* ATCC 25922) were included for comparative purposes. A flow diagram outlining procedures used for selection of final surrogates is shown in Fig. 1.

E. coli isolates were recovered from frozen culture by plating onto Luria-Bertani (LB) agar (Becton Dickinson Diagnostic Systems, Franklin Lakes, NJ) and *E. coli* selective agar plates (mTEC; Becton Dickinson