Food Microbiology 53 (2016) 41–47

Contents lists available at ScienceDirect

Food Microbiology

journal homepage: www.elsevier.com/locate/fm

Conventional curing practices reduce generic *Escherichia coli* and *Salmonella* spp. on dry bulb onions produced with contaminated irrigation water

Alexander W. Emch, Joy G. Waite-Cusic*

Department of Food Science and Technology, 100 Wiegand Hall, Oregon State University, Corvallis, OR 97331, USA

ARTICLE INFO

Article history:
Received 6 May 2015
Received in revised form
4 August 2015
Accepted 5 August 2015
Available online 6 August 2015

Keywords:
Onion
Generic *Escherichia coli*
Salmonella
Greenhouse
Curing
Soils

ABSTRACT

Food Safety Modernization Act (FSMA) has emphasized microbial risks associated with irrigation water. Treasure Valley (eastern Oregon/western Idaho) has the highest yield of dry bulb onions in the country; however, their irrigation water is often non-compliant with current industry and proposed federal standards for fresh produce. Conventional curing practices may provide a mechanism to mitigate irrigation water quality to comply with FSMA regulations. Dry bulb onions were grown in Owyhee silt loam and Semiahmoo muck soils in greenhouses and irrigated with water containing a cocktail of rifampicin-resistant generic *Escherichia coli* and *Salmonella* spp. (4.80 log CFU/ml). To mimic conventional practices, mature onions remained undisturbed in soil without irrigation for 12 days prior to being lifted and cured for 16 additional days. Surviving generic *E. coli* and *Salmonella* spp. were selectively enumerated on using standard plating (Hektoen Enteric Agar with rifampicin; HE + rif) or most probable number (lactose broth with rifampicin; HE + rif) methods. Generic *E. coli* and *Salmonella* spp. on onions decreased 0.19 −0.26 log CFU/g·d during the initial 12 days of finishing. At lifting, generic *E. coli* and *Salmonella* spp. had been reduced to <1 CFU/g and persisted through the end of curing. This study demonstrates conventional curing practices as an effective mitigation strategy for dry bulb onions produced with water of poor microbiological quality.

1. Introduction

Fresh produce is increasingly recognized as a source for foodborne outbreaks due to microbial contamination close to harvest. CDC outbreak data indicates that, from 1998 to 2008, 46% of foodborne illnesses have been associated with an individual commodity or produce category (Painter et al., 2013). Studies have found that the most common sources for microbial contamination in farm-to-table production stem from irrigation water, runoff water from livestock farms, manure, wash water, animal fertilizers, and wildlife (Beuchat and Ryu, 1997; Gelting et al., 2011; Jenkins et al., 2015; Tauxe et al., 1997). Reviews of outbreaks of foodborne illness in the U.S. found that *Salmonella* was the most commonly reported bacterial pathogen, accounting for approximately half of the reported outbreaks of bacterial illnesses (Centers for Disease Control and Prevention, 2010; Scanlan et al., 2011; Sivapalasingam et al., 2004).

The Food Safety Modernization Act (FSMA) was signed into law by President Obama in early 2011 to address a myriad of food safety issues. Following the passage of the FSMA, the Food and Drug Administration (FDA) published proposed rules for the Standards for the Growing, Harvesting, Packing and Holding of Produce for Human Consumption (The Produce Rule) that would drastically impact agricultural production around the country (Food and Drug Administration, 2013). A major portion of the rule focuses on the microbiological quality of irrigation water used for produce that is typically consumed raw ("covered produce"). The proposed rule mandates testing agricultural water for generic *Escherichia coli* levels. Generic *E. coli* is a common indicator of fecal contamination and potential presence of pathogens. A supplemental notice of proposed rulemaking for the Produce Rule was published in September 29, 2014 (Food and Drug Administration, 2014). The supplemental notice proposed to require that producers test and evaluate the microbiological quality of their agricultural water by creating a water quality profile (WQP) that provides a characterization of risk based on two calculated values: the geometric mean

* Corresponding author.
E-mail address: joy.waite-cusic@oregonstate.edu (J.G. Waite-Cusic).

http://dx.doi.org/10.1016/j.fm.2015.08.004
0740-0020/

(GM) and the statistical threshold value (STV) (Food and Drug Administration, 2014). Producers must continue to monitor the microbiological quality of their water to ensure that their WQP accurately describes their water supply. If the water source does not meet the GM or STV criteria, producers must discontinue use of that water source or apply a mitigation strategy (water treatment, irrigation-to-harvest interval, irrigation-to-end of storage interval) to reduce the microbial load before resuming use of the water.

The Treasure Valley growing region of eastern Oregon and western Idaho is an agricultural production area with limited water resource options that rely on a complex irrigation canal system coupled with reclamation and reuse. The combination of open irrigation canals and ditches along with the reclamation of water after passing through fields leads to unpredictably high levels of generic *E. coli*, occasionally >2500 MPN/100 ml (Shock et al., 2013). This region includes approximately 150 growers that farm over 20,000 acres of high yield dry bulb onions (740–760 cwt/acre) (Shock et al., 2000; USDA Economic Research Service, 2011a and 2011b). Dry bulb onions fall under the "covered produce" definition of the Produce Rule; therefore, growers must comply with the water quality standards described above. Producers may mitigate the microbial risk of their water by determining a time interval between the last irrigation and harvest that would achieve a calculated reduction of the initial microbial load to below the mandated GM and STV criteria. In the supplemental to the proposed rule, this mitigation strategy can be applied using a microbial decay rate of −0.5 log CFU/g·d, based on data from previous field trials in leafy greens (Snellman et al., 2014). Conventional finishing practices for dry bulb onion production include an essential extended period of time between last irrigation and harvest. The primary objective of this study was to evaluate conventional onion curing methods as an effective mitigation strategy to reduce selected generic *E. coli* and *Salmonella* applied via contaminated irrigation water. A secondary objective was to compare the survival of generic *E. coli* and/or *Salmonella* on onions produced in two soil types used in commercial onion production in Oregon.

2. Materials and methods

2.1. Greenhouse setup

Two OSU greenhouses (West 6–5 – 700 sq. ft.; West 7–6 – 340 sq. ft.) were used for the onion production studies. Both greenhouses were constructed of solid concrete floors with steel mesh grid tables (5′ × 15′). Greenhouse temperatures were maintained by thermostatic control (High: 24 °C; Low: 10 °C) and temperatures were recorded using a weather station throughout the study (Easyweather Proweather station, Tycon Power Systems, Bluffdale, UT). Large grow trays (4′ × 4′; Botanicare, Chandler, AZ) were placed on each table to collect and contain contaminated runoff. Polyvinyl chloride pipe cages with mosquito netting were constructed around each tray to prevent flying insects from accessing contaminated plants and soil. The Institutional Biosafety Committee (IBC) at Oregon State University was actively engaged in designing suitable containment measures to minimize exposure and release of pathogenic *Salmonella* spp. from the greenhouse environment. Approval from the IBC was granted with the implementation of limited access (locked doors), appropriate signage, personal protection equipment (PPE; lab coats, shoe coverings, face shields), and physical containment of plants (mosquito netting, trays). All inoculated materials used in the greenhouse study (onions, soil, containers, netting, etc) were to be decontaminated by standard autoclaving procedures for biological waste. Upon completion of the study, the runoff containment trays, tables, walls, and floor were sanitized using a pump sprayer filled with a disinfectant solution per manufacturer's instructions (Roccal-D, Pfizer, New York, NY).

2.2. Soil preparation

Soils (~110 kg/soil type) were transported from a commercial onion field in the Willamette Valley (Semiahmoo muck soil) and from the Malheur County Agricultural Experiment Station in the Treasure Valley (Owyhee silt loam) into the Oregon State University greenhouses. Soil was prepared by hand grinding through wire mesh grid boxes (approximately 1.2 cm grid). Two-gallon injection molded planting pots (Gro Pro, Sunlight Supply, Inc., Vancouver, WA) were lined with synthetic cheesecloth (Dairy Connection, Madison, WI) to prevent soil erosion and filled with approximately 3500 g of soil. Prepared pots were distributed into the trays (17–19 pots/tray) throughout both greenhouses. Samples of both soil types (100 g × 3 replicates) were submitted to the Central Analytical Laboratory at Oregon State University for pH and mineral composition analysis.

2.3. Onion production and finishing

Spanish yellow dry bulb onions (Ovation variety; Nunhems USA, Parma, ID) were transplanted directly into pots. Young onion plants had been previously grown from seed (Sakata Seed Company, Morgan Hill, CA) in Buckeye, AZ. Each tray of onion plants (17–19 plants per tray) was treated as a block for a given treatment (inoculated/uninoculated, soil type) trays (A–X) were randomized across both greenhouses. Each onion plant was watered with 200 ml of municipal water in the morning every 2–3 days, as needed. Onion plants were fertilized with OmegaGrow 5-1-1 Organic Liquid Fertilizer (Dixondale Farms, Carrizo Springs, TX) per manufacturer's instructions during week 4 and week 5 after planting. After 5 weeks of growth, irrigation was transitioned to well water (private residence, Philomath, OR) for the remainder of the growing period to eliminate the potential negative impact of chlorinated water on the survival of the inoculum. Irrigation was ceased when onions were determined to be fully mature based on browning and drooping of the onion leaf stalks. To finish the onions, plants remained undisturbed in soil for 12 days and then lifted and set on soil surface to cure for an additional 16 days.

2.4. Bacterial strain, culture conditions, and preparation of inocula

Generic *E. coli* strains (LJH-1247, LJH-1612, LJH-1613) and *Salmonella* spp. (Montevideo LJH-614, Michigan LJH-615, Saintpaul LJH-1262) were used in the inoculation cocktail. Generic *E. coli* strains had been previously isolated by Trevor Suslow's laboratory (University of California–Davis) from lettuce, irrigation water, and soil from the Salinas Valley and were adapted to be rifampicin-resistant by the Linda Harris laboratory (University of California–Davis). *Salmonella* strains were originally isolated from clinical or food samples associated with produce outbreaks. These isolates had previously been adapted to be resistant to rifampicin by the Harris laboratory.

Stock cultures were stored at −80 °C in Tryptic Soy Broth (TSB; Neogen, Lansing, MI) with 40% glycerol. Frozen cultures of each strain were activated by transferring to TSB with incubation at 37 °C for 24 h. For each strain, 0.1 ml of overnight culture was spread onto each of three separate Tryptic Soy Agar plates (TSA, Neogen) containing rifampicin (50 mg/L; Alfa Aesar, Ward Hill, MA; TSA + rif) and incubated at 37 °C for 22–26 h. Bacterial lawns were harvested by adding 3 ml of 0.1% peptone water and scraping with a disposable cell spreader. Cell suspensions for each strain were collected separately and transferred to individual 15 ml sterile conical tubes.