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Inactivation with UV-radiation and internalization assessment of coliforms and *Escherichia coli* in aquaponically grown lettuce

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A B S T R A C T

Escherichia coli O157:H7 can internalize in produce. Therefore, a food safety risk exists for lettuce grown using mixed water sources such as aquaponics, which combines aquaculture with hydroponics. Objectives were to determine antimicrobial efficacy of UV-radiation on reduction of *E. coli*/coliform in a pilot-scale demonstration aquaponic system and assessment for possibility of *E. coli*/coliform internalization in aquaponically-grown lettuce. Water from aquaculture and hydroponic was sampled for 6 weeks prior to lettuce harvest. Water and lettuce samples were spread-plated on 3-M petrifilm™ EC (*E. coli*/coliform) and on m-Endo agar. The average bacterial counts in aquaculture raceways were approximately 10^2 CFU/ml and 10^4 CFU/L on 3-M petrifilm and m-Endo agar, respectively. Bacterial counts did not ($p > 0.05$) increase in the aquaculture raceways or hydroponic greenhouse, suggesting that neither fish nor plants contributed to bacterial growth. The coliform bacteria detected in the aquaponic system reflected their normal presence in the environment where the aquaponic system was located. UV treatment reduced ($p < 0.05$) bacterial counts by approximately 1.5 and 3.0-log on 3-M petrifilm and m-Endo agar, respectively. Internalized coliforms or *E. coli* were not detected in lettuce. This study demonstrates the effectiveness of coliform inactivation by UV-radiation in a pilot-scale real-life aquaponic system.

1. Introduction

Outbreaks of foodborne illnesses traced back to fresh produce and associated with human enteric pathogens have been increasing in recent years (Deering, Mauer, & Pruitt, 2012; Macarisin, Patel, & Sharma, 2014). Foodborne outbreaks associated with produce as a proportion of all foodborne outbreaks has increased from 0.7% in the 1970's to over 6% in the 1990's (Sivapalasingam, Friedman, Cohen, & Tauxe, 2004). In a case study reviewing overall outbreaks of *E. coli* O157:H7 in the United States between 1982 and 2002, 350 reported outbreaks resulted in 8598 confirmed cases (Rangel, Sparling, Crowe, Griffin, & Swerdlow, 2005). Transmission routes for 183 of the 350 overall outbreaks were determined to be food borne of which 21% were linked to produce (Rangel et al., 2005).

Fecal contamination resulting in foodborne outbreaks involving produce is of particular concern. This includes the contamination of produce with the enteric pathogen *Escherichia coli* O157:H7. Epidemiological data indicate that 8.3% of cattle may asymptomatically shed *E. coli* O157:H7 in feces (Solomon, Yaron, & Matthews, 2002). Therefore, one transmission vehicle for this foodborne pathogen is surface water run-off contaminated with cattle feces.

Aquaponics is a novel and growing industry that combines the production of plants with fish using hydroponics and aquaculture, respectively (Timmons & Ebeling, 2007). The effluent water from the aquaculture system where the fish are grown is a source of nutrients for plants grown in the hydroponic system. In general, total and/or fecal coliform are typically used as indicators for fecal contamination in food systems. The risk of fecal contamination in recirculating aquaponic systems that exclude warm-blooded animals and use potable water should be relatively low. Although the body temperature of fish is too low (i.e., cold-blooded animals) to support proliferation of enteric pathogens to high numbers, fish have been shown to harbor foodborne pathogens such as *E. coli* O157:H7 when contaminated with feces from warm-blooded animals (Al-Harbi, 2003). The fish may even carry these enteric bacteria in their intestines for up to 7 days (Geldreich & Clarke, 1966). Supporting evidence indicates that coliforms within the gut of fish are not permanent flora, but instead they correlate with the level of contamination within the fish environment (Geldreich & Clarke, 1966). A high level of coliform detection in water provides evidence that there may be fecal contamination by a warm-blooded animal, indicating a

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risk for enteric pathogens to be present. The detection of fecal contamination in aquaponic operations has been predominantly seen in systems using water source of poor quality and/or allow for fecal inputs from domestic animals or wildlife (Fox et al., 2012). Produce grown in systems such as these, may potentially be at risk for enteric pathogen contamination through internalization, rendering efforts for surface decontamination/sanitation inadequate.

E. coli O157:H7 has been shown to have several ways to internalize within the tissue of produce (Gomes et al., 2009). For example, evidence supports that *E. coli* O157:H7 cells can penetrate the stomata and junction zones of lettuce leaves after being cut as well as be transported into the plant through the root system. These modes of pathogen internalization leave post-harvest washing and surface decontamination/sanitation inadequate (Solomon et al., 2002; Jablasone, Warriner, & Griffiths, 2005). Of particular significance to the present study, it also has been shown that *E. coli* O157:H7 can be transported through the roots of hydroponically grown spinach and into the edible portions of the plant (Macarisin et al., 2014).

The main objective of the present study was to determine the antimicrobial efficacy of UV-radiation on reduction of *E. coli*/coliforms in aquaculture effluent water supplying nutrients to grow lettuce hydroponically on a floating raft. A possibility for internalization of *E. coli*/coliform through the roots of lettuce in the hydroponic system with sustained fecal contamination was also investigated.

2. Materials and methods

2.1. Aquaponic facility

A cold-water, spring-fed hydroponic greenhouse, part of the spring-fed, gravity-flow-through raceway aquaculture demonstration facility growing trout, was used in the present study. Spring water was collected as surface run-off from the surrounding cattle pastures and flowed by gravity through trout raceways before being pumped into the hydroponic greenhouse. The water supply to the aquaculture facility received run-off from cattle pastures allowing for fecal contamination from both cattle and wildlife.

The aquaculture facility is shown in Fig. 1. The spring water first flowed into a head-box before entering the trout raceways. The head-box did not contain any fish and was located at a slightly higher elevation than trout raceways; therefore, the head-box delivered gravity flow to raceways. The raceway system was composed of four paired units with water flowing in a serial fashion through each of four units. Each raceways unit was approximately 10 m long, 1 m wide with 1 m deep water. The water flow rate in each raceway unit was approximately 662 L/min. Thus, there were a total of eight raceways where rainbow trout (*Oncorhynchus mykiss*) were reared. Each of the eight raceways had a quiescent zone from which fish were excluded to allow settling of solid waste from fish. Solid waste was periodically removed from the quiescent zone and by-passed the hydroponic system. Effluent leaving the last raceways flowed into a tail-box and out from the aquaculture system to the hydroponic greenhouse.

The hydroponic greenhouse is shown in Fig. 1. It housed three lined plywood structures, each with three channels whose base was approximately 40 cm above the ground. Eight channels were used in the present study. The ninth channel was used for water overflow. Each channel was approximately 39 cm wide and 238 cm long. It held water at a depth of approximately 20 cm. Each channel was scrubbed clean and disinfected with chlorine prior to the study. Effluent from the aquaculture system tail-box was continuously pumped into each of the nine channels through an inlet controlling flow volume at approximately 7.6 L/min. Water drained from each channel through a standpipe drain. Four of the eight channels were randomly selected to receive UV-treated water. The in-line UV-light system (described below) was installed so that it would treat the aquaculture effluent water between the tail-box and inlet to the hydroponic greenhouse. In order to assess antimicrobial efficacy log reductions of total *E.coli*/coliforms between UV-treated and untreated water entering the hydroponic greenhouse, as well as the mean difference between the head-box and tail-box in the aquaculture system were determined.

Lettuce (*Latuca sativa* "Red Sails") was grown in polystyrene Speedling® trays. Vermiculite was used as a growing medium for lettuce. Seeds were sown directly on to the vermiculite surface using a vacuum seeder. One tray held 128 individual cells to grow lettuce. Each tray was approximately 34 cm wide and 67 cm long. Three trays floated end-to-end on the water surface of each channel between the inlet and the standpipe drain.

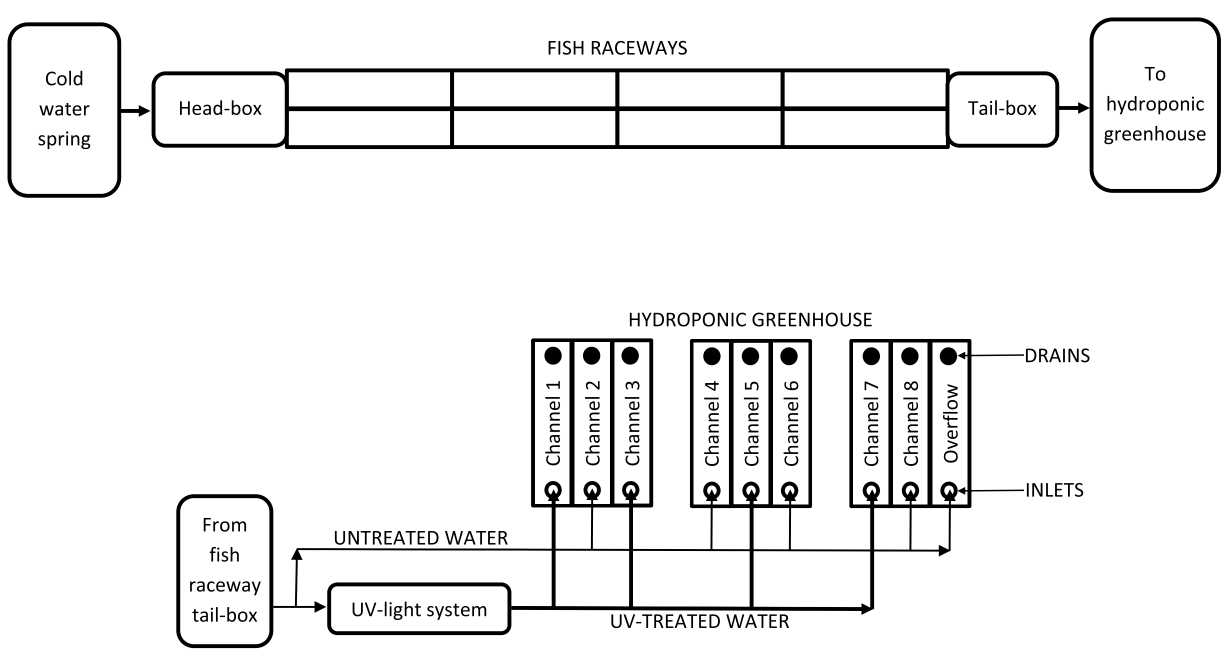

Fig. 1. A schematic diagram of the aquaponic system used in the present study consisting of aquaculture raceways combined with hydroponic greenhouse and an in-line UV-radiation system.