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Monitoring generic *Escherichia coli* in reclaimed and surface water used in hydroponically cultivated greenhouse peppers and the influence of fertilizer solutions

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ABSTRACT

Systematic monitoring of indicator microorganisms, such as *Escherichia coli*, can help to identify potential risk factors for faecal contamination in the agricultural environment. In this study, levels of *E. coli* in irrigation water (both reclaimed and surface water), water sprayed in humidifiers to regulate ambient humidity, and pepper fruits were assessed in a commercial greenhouse of hydroponically cultivated crops. Additionally, the role of fertilizer solutions as a potential vector of contamination was investigated. Lab-scale studies were also performed to evaluate the influence of fertilizer solutions on the growth/survival of *E. coli* in irrigation water. As expected, higher levels of *E. coli* were detected in reclaimed water compared with surface water. No link between *E. coli* prevalence in irrigation water and presence in fruit could be established. Regarding the fertilizer solutions, *E. coli* was detected more frequently and in higher levels in the fertilizer solution richer in micronutrients. Low concentrations of *E. coli* were also present in pulverized water sprayed inside the greenhouse to control humidity. In lab-scale experiments, *E. coli* showed potential for surviving but not for growing in most fertilizer solutions and irrigation water. Fertilizer solution of HNO_3, was the only solution in which no *E. coli* were able to survive in the irrigation head and a rapid inactivation was observed in lab-scale tests. These results suggest that there is a low risk of contamination in this agricultural system despite the combination of higher risk irrigation water sources (reclaimed and surface water) and the hydroponic growing system. Nevertheless, special care should be taken regarding the microbiological quality of the agricultural solutions in direct contact with the edible parts of the crop.

1. Introduction

Reclaimed water is used in agriculture in arid and semi-arid regions of the world as a way to overcome water scarcity (Becerra-Castro, Lopes, Vaz-Moreira, Silva, Manaia, 2015). However, there are concerns related to the microbiological quality of reclaimed water and its possible consequences on food safety (Olivieri, Seto, Cooper, Cahn, Colford, 2014). Even though irrigation water contact with edible plant parts is avoided, survival of pathogenic bacteria in agricultural substrates and potential internalization through the roots are relevant issues to be taken into account in agricultural use of reclaimed water (Bernstein, 2011).

Monitoring sampling programs are usually based on the detection of specific pathogens. However, there is an increased interest in using microbial indicators to characterize microbial contamination in the environment of primary production to overcome current limitations associated with the pathogen detection such as low prevalence and high cost (Mukherjee, Speh, & Diez-Gonzalez, 2007; Park, Navratil, Gregory, Anciso, & Ivanek, 2013). Nonetheless, reliability of indicator microorganisms can be affected by different factors such as climatic conditions or the use of agrochemicals (Pachepsky, Shelton, McLain, Patel, & Mandrell, 2011; Castro-Ibáñez, Gil, Tudela, Ivanek, & Allende, 2015).

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Hydroponic cultivation in greenhouses is supposed to reduce the number of vectors that can contaminate the crops with pathogenic bacteria (Orozco, Rico-Romero, & Escartín, 2008a). However, there are still different vectors (e.g. irrigation water) that can be the entry point of pathogenic bacteria in this agricultural system (Orozco et al., 2008b). Once in the environment, pathogenic microorganisms are very difficult to eliminate (Allende & Monaghan, 2015). Stanghellini and Rasmussen (1994) described that peat, water source and insects are potential vectors for the contamination of nutrient solution in hydroponic systems. The safety issues associated with the use of reclaimed water have been evaluated in tomatoes, and results suggested that urban wastewater in combination with a production system that minimizes contact with the edible part of the crop does not represent a microbial risk (López-Gálvez, Allende, Pedrero-Salcedo, Alarcon & Gil, 2014). The *Codex Alimentarius* (2007) highlights that plants grown in hydroponic systems absorb nutrients and water, which constantly change the composition of the nutrient solution. Because of this, Codex recommends that water used in hydroponic culture should be changed frequently, or if recycled should be treated to minimize microbial contamination (Allende & Monaghan, 2015).

There is some information available on the behaviour of plant pathogens in nutrient solutions used in hydroponic systems (Vallance, Déniel, Le Floch, Guérin-Dubrana, Blancard, 2011), but little about the potential of such solutions to sustain the survival of faecal indicators or human pathogenic microorganisms (Settani, Miceli, Francesca, Cruciata, & Moschetti, 2013). Staley, Rohr, and Harwood (2010) observed that the addition of agrochemicals, including inorganic fertilizers, affected the levels of fecal indicator bacteria in irrigation water. To gain insights regarding the microbial risk associated with water sources in a commercial greenhouse for hydroponically grown peppers, the present study focused on the evaluation of *Escherichia coli* prevalence in two types of irrigation water, reclaimed and surface water, as well as in the peppers and the impact of fertilizer solutions.

2. Materials and methods

2.1. Experimental design and growing conditions

Bell pepper plants (*Capsicum annuum* L. cv Tamarin) were grown in a commercial greenhouse located in Balsicas (Murcia, Spain) from December 2013 until August 2014. Details related to the greenhouse location, climatological monitoring and acquisition data, irrigation water characteristics and crop management were previously described (Lopez-Galvez et al., 2014). Two types of water sources were used for irrigation: reclaimed water (Reclaimed) from the tertiary treatment effluent of a wastewater treatment plant (Roldán-Balsicas, Murcia, Spain), and surface water (Surface) from an Irrigation Community. Irrigation water was supplemented with fertilizer solutions as needed to obtain the fertilized irrigation water (Fertilized Reclaimed and Fertilized Surface Water). Five different fertilizer solutions (FS) were used: **FS1** (Peak® MKP 0-52-34, 75 g/L KH_2PO_4, ICL Fertilizers, Beer Sheva, Israel); **FS2** (Multi-K GG, 75 g/L KNO_3, Haifa Chemicals, Haifa, Israel); **FS3 (**YaraLiva Calcinit, 75 g/L $Ca(NO_3)_2$, Yara Iberian, Madrid, Spain); **FS4** (Biomad micro®, 8 mL/L micronutrients solution, Agroquímicos Los Triviños, Murcia, Spain + Bastion®, 0.5 g/L Fe, Agroindustrial Kimitec, Almería, Spain); and **FS5** (40 mL/L HNO_3 54%, Agroquímicos Los Triviños, Murcia, Spain). Nutrients were supplied to the plants as required through the irrigation water. Five different fertilizing solutions prepared in individual tanks using surface water were used to this end. In all cases, coconut fibre (Pelemix, Alhama de Murcia, Spain) was used as substrate for the hydroponic system. A total of 120 plants were divided into two treatments depending on the irrigation water (Reclaimed and Surface treated plants) with 3 replicates of 20 plants each. During the growing period, minimum and maximum temperatures inside the greenhouse were 12.3 °C and 28.2 °C, respectively, with an average of 21.0 ± 3.9 °C. The relative humidity (RH) in the greenhouse ranged from 55.8% to 99.9% with an average of 79.2 ± 7.6%. The total amount of irrigation water applied was 5022 and 5012 m^3/ha for reclaimed and surface water, respectively.

2.2. Sampling points for water and fertilizer solutions

E. coli prevalence and concentration were monitored in samples (2 L) of irrigation water before (Reclaimed and Surface) and after fertilization (Fertilized Reclaimed and Fertilized Surface). The drainage obtained from the hydroponic substrate lines was another sampling point where water was tested (Drainage Reclaimed and Drainage Surface). Samples were also taken from the different fertilizer solution tanks (FS1-FS5). Atomized water from the nozzles to control RH inside the greenhouse was also evaluated. Sampling was performed 1–2 times per week during 16 weeks from April 28th until August 12th 2014, with a total of 240 water samples. *E. coli* analyses were carried out as previously described (Lopez-Galvez et al., 2014).

2.3. Microbiological analysis of peppers

Collection of pepper samples was performed every 2 weeks during the harvest period, from May until August 2014, for a total number of 222 samples over 8 sampling times. At each sampling time, 10–15 samples were taken from each growing condition, which corresponded to peppers irrigated with reclaimed and surface water. Green peppers grade U.S. No. 1 or grade U.S. Fancy, as defined in the United States standards for grades of sweet peppers (USDA., 1989), were randomly picked from the plants at a height of 0.25–1.5 m above the surface of the substrate and transferred aseptically into sterile bags. For the analysis of each sample, pepper flesh was diced in pieces of approximately 3 × 3 cm using a stainless steel knife under aseptic conditions. Samples of 25 g of pepper were taken randomly and diluted 1:5 in buffered peptone water (20 g/L; Scharlab, Barcelona, Spain) and then homogenized using a stomacher for 1 min. Homogenized samples were used for direct plating, filtration, and enrichment. For direct plating, 1 mL of sample was poured in a petri plate and mixed with melted agar before incubation. In order to reduce the detection limit, 25 mL of homogenized sample was filtered through a 0.45 μm membrane filter (Sartorius, Madrid, Spain). The remaining contents of the bags were incubated at 37 °C for 18–24 h for enrichment of viable populations. After enrichment, fluids were applied to the surface of Chromocult coliform agar (Merck, Darmstadt, Germany) with a bacteriological loop and were incubated at 37 °C for 24 h before interpretation of results.

2.4. Survival and growth of E. coli in irrigation water and fertilizer solutions

Plate count experiments were performed in triplicate to determine potential survival or growth of *E. coli* in the irrigation water types (Reclaimed and Surface) as well as in the different fertilizer solutions (FS1 to FS5) using a lab-scale test where reclaimed and surface water were used for the preparation of the solutions. A cocktail of three *E. coli* strains (CECT 471, 515, and 516) was prepared by mixing cells washed twice by centrifugation (4500 *g*, 10 min) in sterile distilled water after overnight incubation (BHI, 37 °C, 20 h). The *E. coli* strains were inoculated in the irrigation water with and without fertilizer solutions to reach a level of 2 log