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Safety assessment of greenhouse hydroponic tomatoes irrigated with reclaimed and surface water

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ABSTRACT

The impact of reclaimed and surface water on the microbiological safety of hydroponic tomatoes was assessed. Greenhouse tomatoes were irrigated with reclaimed and surface water and grown on two hydroponic substrates (coconut fiber and rock wool). Water samples (n = 208) were taken from irrigation water, with and without the addition of fertilizers and drainage water, and hydroponic tomatoes (n = 72). Samples were analyzed for indicator microorganisms, generic *Escherichia coli* and *Listeria* spp., and pathogenic bacteria such as *Salmonella* spp. and Shiga-toxigenic *E. coli* (STEC), using multiplex real-time PCR (RT-PCR) after enrichment. The correlation between climatological parameters such as temperature and the levels of microorganisms in water samples was also determined. In irrigation water, generic *E. coli* counts were higher in reclaimed than in surface water whereas *Listeria* spp. numbers increased after adding the fertilizers in both water sources. In drainage water, no clear differences in *E. coli* and *Listeria* numbers were observed between reclaimed and surface water. No positive samples for STEC were found in irrigation water. Presumptive positives for *Salmonella* spp. were found in 7.7% of the water samples and 62.5% of these samples were reclaimed water. *Salmonella*-positive samples by RT-PCR could not be confirmed by conventional methods. Higher concentrations of *E. coli* were associated with *Salmonella*-presumptive positive samples. Climatological parameters, such as temperature, were not correlated with the *E. coli* and *Listeria* spp. counts. Tomato samples were negative for bacterial pathogens, while generic *E. coli* and *Listeria* spp. counts were below the detection limit. The prevalence of presumptive *Salmonella* spp. found in irrigation water (reclaimed and surface water) was high, which might present a risk of contamination. The absence of pathogens on greenhouse hydroponic tomatoes indicates that good agricultural practices (GAP) were in place, avoiding the microbial contamination of the fruit.

1. Introduction

Tomatoes have been associated with foodborne outbreaks (Hanning et al., 2009; Hedberg et al., 1999). The combination of *Salmonella enterica*-tomato was the second pathogen-commodity pair with a higher risk in a ranking elaborated using data from US and EU outbreaks (Anderson et al., 2011; EFSA, 2013). Previous investigations of *Salmonella* outbreaks due to contaminated tomatoes highlighted contaminated irrigation water and contaminated wash water as sources (Hanning et al., 2009). Contamination via fecally-contaminated irrigation water could expose tomatoes to contamination with pathogens such as *Salmonella* if there is an influence from sewage effluent contamination, and particularly if the water is delivered by spray irrigation. *Listeria monocytogenes* and Shiga-toxigenic *Escherichia coli* (STEC) have also been detected in fresh tomatoes (Gómez-Aldapa et al., 2013; Mérida et al., 2009; Pingulkar et al., 2001). However, irrigation with contaminated water does not always result in contamination of harvested edible tomatoes. Jablasone et al. (2004) demonstrated that water contaminated with *Salmonella* applied directly into the soil by drip irrigation did not result in the transmission of *Salmonella* to tomato fruit.

Within the EU, most of the countries cultivate tomatoes in greenhouses on different soilless substrates (principally rock wool) which can be coupled or not with a central hot water heating system, and computerized control of environmental conditions and watering (EFSA, 2013). Greenhouse cultivation is assumed to be safer than open field from the microbiological point of view due to the minimization of some risk factors associated with sources of preharvest contamination (L. Orozco et al., 2008). For both cultivation systems, one of the most important potential sources of contamination with hazardous bacteria is irrigation water and water used for foliar-applied crop management treatments. This is particularly critical in greenhouse cultivation because of the lack of solar radiation, which has been described as one of the factors responsible for the decrease of bacterial contamination in the environment (Castro-Ibáñez et al., 2014; Whitman et al., 2004). Extensive data available in the literature shows that irrigation water for fresh produce can be a source of pathogenic bacteria such as *Salmonella*,

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pathogenic *E. coli* and *L. monocytogenes* (Holvoet et al., 2014; Pachepsky et al., 2011; Soderstrom et al., 2008; Steele and Odumeru, 2004). Within the different types of water used for irrigation, the highest concern is related to the use of untreated or improperly treated wastewater (Pachepsky et al., 2011). Regarding irrigation techniques, drip irrigation is considered to be safer compared to those others which allow direct contact between the irrigation water and the edible part of the produce such as sprinkle irrigation (Oron, 2002). However, pathogenic bacteria can internalize through the roots and survive in crops, although exposure to high levels of pathogens is needed for this phenomenon to occur, and even if it occurs, survival into the plant might be limited (Erickson, 2012).

While in some studies the presence of foodborne pathogenic bacteria on hydroponic greenhouse grown tomatoes has not been detected (Luedtke et al., 2003), other studies described the presence of *Salmonella* spp. and pathogenic *E. coli* in this type of crop management system (L. Orozco et al., 2008; R.L. Orozco et al., 2008). These results show that in spite of the lower risks associated with greenhouse drip irrigated crops, pathogenic bacteria could reach hydroponic greenhouse tomatoes in different ways (R.L. Orozco et al., 2008). The aim of the present study was the safety assessment of hydroponic tomatoes grown in a greenhouse and irrigated with different water sources, reclaimed and surface water, and the establishment of the correlations between climatological factors, such as temperature, and microbial growth in water and produce samples.

2. Materials and methods

2.1. Experimental design

Tomato plants (*Lycopersicon esculentum* Mill. cv Tirade) were grown from January through August 2013 in a greenhouse located next to a wastewater treatment plant (Roldán-Balsicas, Murcia, Spain) (37°47′48″ N, 0°57′33″ W). Climatological data including rainfall, temperature, relative humidity (RH), wind speed and solar radiation were acquired by a weather station located on the upper part of the greenhouse. The RH and temperature inside the greenhouse were monitored. Two types of water were used for irrigation: reclaimed water (RW) from the wastewater treatment plant and surface water (SW) from the Irrigation Community of Campo de Cartagena. Reclaimed water was obtained after treatment of urban wastewater including primary treatment, secondary treatment, and tertiary treatment with ultraviolet (UV) light. Primary treatment usually includes screening to trap solid objects and sedimentation by gravity to remove suspended solids while secondary treatment consist in a processing for treating sewage and wastewaters using air and biological flocculation for the removal of suspended solids, colloids and organic matter present in wastewater (Renault et al., 2009). Surface water was a mix of the Tajo-Segura water transfer and well water.

2.2. Fertigation system and management

The irrigation head was in a shed out of the greenhouse (see SI 1) and consisted of two tanks, one for RW and another for SW before adding fertilizers, other tanks for the fertilizers (monopotassium phosphate (F1), potassium nitrate (F2), calcium nitrate (F3), microelements solution (F4) and nitric acid (F5)) and two tanks after adding the fertilizers (FRW and FSW). Fertilized waters were used for the fertigation of tomato plants. Water flowed from the irrigation head to the greenhouse inside closed pipes. The fertigation was scheduled weekly based on recorded weather data and plant phenological stage. During the entire period, the nutrient solution was adjusted based on the concentration of nutrients for each type of irrigation water, controlling the electrical conductivity (EC) ≤ 2 dS m^{-1}, pH of 5.5 and the volume of drainage between 25–35%. During the crop cycle, the nutrient solution in terms of N_2–P_2O_5–K_2O ranged from 10–1.5–5.5 mmol L^{-1} at the beginning of cultivation (01/01/2013) to 14–1.5–6 mmol L^{-1} at the end of the crop cycle (28/08/2013). Residence time of the water in the tanks decreased from winter to summer. At the beginning of the study, tanks were consumed every 3–4 weeks while in summer they were replenished twice a day.

Two common substrates were used for hydroponics, coconut fiber (Pelemix, Alhama de Murcia, Spain) and rock wool (Grodan, El Ejido, Spain). Four different growing conditions defined by the types of irrigation water and hydroponic substrate were evaluated: irrigation with reclaimed water in coconut fiber (RWCF), surface water in coconut fiber (SWCF), reclaimed water in rock wool (RWRW) and surface water in rock wool (SWRW). Three replicates of 15 plants for a total of 45 plants per treatment were grown (see SI 2).

2.3. Growing conditions

During the growing period (January–August 2013), the minimum and the maximum temperatures inside the greenhouse were 15.0 °C and 29.1 °C, respectively. RH in the greenhouse ranged from 55% to 97% with an average of 78%. The total amount of applied irrigation water was 16,566; 17,370; 17,000 and 17,227 m^3 ha^{-1} for RWCF, SWCF, RERW and SWRW, respectively. These data correspond with representative hydroponic tomato water consumption in greenhouse production under these climatic conditions (Gallardo et al., 2007).

2.4. Microbiological analysis of water

Water sampling was performed weekly during June, July and August 2013 for a total of 13 weeks ($n = 208$). Irrigation water was sampled before (RW and SW) and after fertilization (FRW and FSW) and it was also collected from the hydroponic substrate lines of coconut fiber and rock wool as drainage water (RWCF, SWCF, RWRW and SWRW). Duplicate samples of 2-L were taken for each type of water using sterile plastic jars. At each sampling week, two samples per water type ($n = 16$) were analyzed by direct plating for *Listeria* spp. and *E. coli*, while only one sample per water type ($n = 8$) was analyzed by multiplex RT-PCR for *Salmonella* spp. and STEC detection.

2.4.1. Listeria spp.

Filtration and direct plating were used to quantify *Listeria* spp. and *L. monocytogenes*. Volumes between 10 and 100 mL were filtered through 0.45 μm membrane filters (Sartorius, Madrid, Spain) using a filter holder manifold (Millipore, Madrid, Spain). Brilliance Listeria Agar (Oxoid, Basingtoke, UK) was used as culture media for filters and direct plating, incubated at 37 °C for 48–72 h before interpretation of results. Blue/green colonies without white opaque halo were considered as *Listeria* spp. other than *L. monocytogenes* or *Listeria ivanovii*, while colonies with the same color that developed a halo were considered presumptive *L. monocytogenes* or *L. ivanovii*.

2.4.2. E. coli

For quantification of *E. coli* in water samples, filtered and non-filtered samples were plated in Chromocult coliform agar (Merck, Darmstadt, Germany). Plates were incubated for 24 h at 37 °C before interpretation. Dark blue-violet colonies were considered positives for *E. coli*.

2.4.3. STEC and Salmonella spp. detection

For the detection of Shiga-toxigenic *E. coli* and *Salmonella* spp. by multiplex RT-PCR, a variable number of filters depending on filterability of the water were used to filter 1 L of each type of water. The filters were pooled in a stomacher bag and 150 mL of buffered peptone water was added. Bags were incubated for 24 h at 37 °C for enrichment, and then a volume of 7 mL was decanted into 15 mL centrifuge tubes and stored at −20 °C with 30% glycerol. For the RT-PCR analyses of water samples ($n = 104$), the *Salmonella*/O157 and STEC screening GeneDisc Pack was