PAPER

RECOVERY AND BEHAVIOUR OF STRESSED *ESCHERICHIA COLI* O157:H7 CELLS ON ROCKET LEAF SURFACES INOCULATED BY DIFFERENT METHODS

ANAS A. AL-NABULSI[1*], AMIN N. OLAIMAT[2], TAREQ M. OSAILI[1], HEBA M. OBAIDAT[1], ZIAD W. JARADAT[3], REYAD R. SHAKER[4] and RICHARD A. HOLLEY[5]

[1]Department of Nutrition and Food Technology, Faculty of Agriculture, Jordan University of Science and Technology, Irbid, Jordan

[2]Department of Clinical Nutrition and Dietetics, Faculty of Allied Health Sciences, Hashemite University, Zarqa, Jordan

[3]Department of Biological and Genetic Engineering, Jordan University of Science and Technology, Irbid, Jordan

[4]Department of Clinical Nutrition and Dietetics, University of Sharjah, Sharjah, United Arab Emirates

[5]Department of Food Science, Faculty of Agriculture and Food Science, University of Manitoba, Winnipeg, Canada

*Corresponding author: Tel. +962 02 7201000, Fax +962 02 7201078, email: anas_nabulsi@just.edu.jo

ABSTRACT

E. coli O157:H7 is an emerging public health concern worldwide because of its low infectious dose and ability to survive under adverse conditions. Tests were conducted to determine the ability of unstressed *E. coli* O157:H7 cells or those stressed by acid, cold, salt exposure or starvation to survive on the surfaces of rocket leaves after contamination by three methods (dip, spray or spot inoculation) and following storage at 10 or 25°C. *E. coli* O157:H7 numbers recovered from rocket leaves contaminated by the different techniques were in the order of dip > spot > spray inoculation. Numbers of stressed *E. coli* O157:H7 recovered after inoculation by all three methods increased significantly over 7d storage at 10 or 25°C, while unstressed *E. coli* O157:H7 only grew following dip inoculation. Exposure to adverse environmental conditions may increase the risk of *E. coli* O157:H7 survival and spread on leafy green vegetables.

- Keywords: *E. coli* O157:H7, environmental stress, leafy green vegetables, rocket leaves, inoculation method -

INTRODUCTION

Escherichia coli O157:H7 is a facultatively anaerobic, Gram-negative rod-shaped enteric bacterium which produces Shiga toxins 1 and/or 2 as important virulence factors and emerged as a foodborne pathogen in 1982. *E. coli* O157:H7 infection presents with numerous symptoms including abdominal pain, watery and bloody diarrhea, vomiting, mild fever, sometimes leading to hemorrhagic colitis and the hemolytic uremic syndrome (HUS) with renal tubular damage (WHO, 2011). SCALLAN *et al.* (2011) and THOMAS *et al.* (2013) reported that *E. coli* O157:H7 is associated with 63,153 and 12,827 foodborne illnesses every year in the US and Canada, respectively. *E. coli* O157:H7 illness outbreaks have been associated with a variety of foods including ground beef, spinach, lettuce, radishes, vegetable sprouts, fermented sausages, unpasteurized fruit juices, apple cider and raw milk (CHAURET, 2011). Annually, fresh produce is responsible for 45.9% of foodborne illnesses, 38.1% of hospitalizations and 22.9% of deaths caused by contaminated food in the US (PAINTER *et al.*, 2013). Furthermore, *E. coli* O157:H7 has been associated with repeated illness outbreaks resulting from the consumption leafy vegetables including lettuce, spinach, parsley and rocket leaves (OLAIMAT and HOLLEY, 2012; NYGARD *et al.*, 2008).

In recent years contamination of raw or minimally processed food products such as fresh produce with *E. coli* O157:H7 has become a concern worldwide because of its low infectious dose and ability to survive for long periods in the environment (CHAURET, 2011; DELAQUIS *et al.*, 2007). This contamination may occur on the surface of leafy vegetables due to the transfer of the pathogen from soil or water (DELAQUIS *et al.*, 2007). Survival of *E. coli* O157:H7 on these surfaces is affected by several factors including nutrient availability, competition with indigenous microflora, UV radiation and relative humidity (BRANDL, 2006). Fresh produce can be contaminated with foodborne pathogens during pre- or post-harvest processes (OLAIMAT and HOLLEY, 2012). Agricultural production and post-harvest environments may exert a variety of stresses on pathogens, which may affect their survival on the final product. In response, strategies have been developed by exposed pathogens to reduce the impact of this stress, including formation of aggregates in protective niches, localization in biofilms, and internalization within plant tissues. Bacteria have also been shown to respond to these stresses through genetic and/or physiological means including stress adaptation, development of cross-protection mechanisms, conversion to a viable but non-culturable (VBNC) state, through heterogeneous phenotypic expression, and by sheer genetic diversity (DINU *et al.*, 2009). Studies have shown that ability of *E. coli* O157:H7 to exhibit responses to sub-lethal environmental stresses, which may enable its survival under more severe conditions, enhance its resistance to subsequent processing conditions, and/or enhance its virulence. Therefore, understanding the effects of environmental stress caused by acid, cold, starvation and abnormal osmotic conditions on the survival of *E. coli* O157:H7 is important in order to assess and minimize the risk of foodborne illnesses caused by this organism (CHUNG *et al.*, 2006).

It has been reported that damaged produce supports the growth of foodborne pathogens, however, intact vegetables like lettuce, tomatoes, endive, carrots, cabbage, asparagus, broccoli and cauliflower may also permit the growth or survival of pathogens (OLAIMAT and HOLLEY, 2012). Several studies have investigated growth and survival of *E. coli* O157:H7 on the surfaces of leafy green vegetables including lettuce (LANG *et al.*, 2004b; MARKLAND *et al.*, 2013; MCEVOY *et al.*, 2009; CHANG and FANG, 2007), parsley (ISLAM *et al.*, 2004; LANG *et al.*, 2004b), spinach (MARKLAND *et al.*, 2013; LUO *et al.*, 2009), basil (MARKLAND *et al.*, 2013) and thale cress (COOLEY *et al.*, 2003). However, little information is available on the survival of stressed *E. coli* O157:H7 cells on the surfaces of leafy greens (MCEVOY *et al.*, 2009).

Since produce contamination may occur during pre- or post-harvest activities, different techniques have been used to reproduce contamination that may occur commercially. Spot inoculation, where a volume of inoculum containing a known cell density is applied at several locations on produce surfaces, can represent contamination that may occur from contact with soil, workers' hands, or equipment surfaces. Dip inoculation can represent contamination that may occur from run-off as well as irrigation, flume water use and water immersion which are common among industry practices. Additionally, spray inoculation can represent contamination that may result from aerosols (BEUCHAT *et al.*, 2003). Given the physical differences among each of the ways produce can become accidentally or deliberately contaminated, it became of interest to determine whether the experimental method used for fresh produce inoculation could influence inoculated pathogen survival or growth. Thus, the objective of the current study was to compare the recovery of unstressed or acid-, cold-, starved- and salt-stressed *E. coli* O157:H7 cells from the surfaces of rocket leaves inoculated by different methods and stored at 10° or 25°C.

MATERIALS AND METHODS

Preparation of bacterial strains and inocula

Four clinical isolates of *E. coli* O157:H7 (00:0304, 02:0627, 02:0628, and 02:3581) now in the Department of Nutrition and Food Technology, Jordan University of Science and Technology culture collection were stored individually at -80°C

in Trypticase Soy Broth (TSB; Oxoid Ltd., Basingstoke, UK) containing 20% (vol/vol) glycerol (Sigma-Aldrich, St. Louis, MO). Frozen stock cultures were activated by transferring one loopful from each culture to 10 mL of TSB and incubating at 37°C for 24 h. The strains were streaked on Sorbitol MacConkey Agar (SMAC) plates and stored at 4°C. One colony was transferred to 10 ml TSB and incubated at 37°C for 24 h. Equal volumes of each strain were mixed to prepare an *E. coli* O157:H7 cocktail which was centrifuged at 4,500 rpm for 20 min, the supernatant fluid was removed and the pellet was washed with sterile deionized water and then transferred to 10 mL of sterile deionized water. This suspension was diluted in sterile deionized water to achieve 10^8 CFU/mL.

Preparation of stressed *E. coli* O157:H7

Acid-stressed cells were prepared by transferring a loopful of each strain to 10 ml of TSB containing 10 g/L glucose and incubated at 37°C for 18 h where ~ 9 log CFU/mL was reached at a final pH 4.9 ± 0.1 (AL-NABULSI *et al.*, 2014; LEENANON and DRAKE, 2001). Equal volumes of each strain were mixed in sterile tubes to prepare a cocktail mixture containing equal numbers of each strain.

Salt-stressed cells were prepared by transferring a loopful from each strain into 10 ml of TSB supplemented with 0.65 M NaCl and incubated at 37°C for 18 h where ~ 9 log CFU/mL was reached. A cocktail was prepared containing equal numbers of each strain as described above and resuspended in 10 ml sterile deionized water (AL-NABULSI *et al.*, 2014; HAJMEER *et al.*, 2006).

Cold-stressed cells were prepared by inoculating a loopful of each strain into 10 ml of TSB at 37°C for 18 h where ~ 9 log CFU/mL was reached. A cocktail was prepared containing equal numbers of each strain as described above, was resuspended in 10 ml TSB and incubated for 7 d at 5°C (AL-NABULSI *et al.*, 2014; LEENANON and DRAKE, 2001).

Starved cells were prepared by inoculating a loopful of each strain into 10 mL of TSB which was incubated at 37°C for 18 h. A cocktail was prepared containing equal numbers of each strain as described above in saline solution (0.85% NaCl, pH 6.6) and incubated further for 48 h at 37°C (AL-NABULSI *et al.*, 2014; LEENANON and DRAKE, 2001).

Inoculation of leaf surfaces by spot, spray or dip methods

Rocket leaves were purchased from a supermarket in Irbid, Jordan on the day of each experiment. Damaged leaves were removed; intact leaves were washed with tap water and dried using a salad spinner. Unstressed and stressed *E. coli* O157:H7 cells were used to inoculate the rocket leaves to obtain an inoculum level of 7.0 log CFU/leaf. The following procedures were used for inoculation of rocket leaves: for spot inoculation, 50 µL cell suspension was added at different places on the surface of each leaf; for dip inoculation leaves were dipped in 100 mL of inocula prepared as described above for 1 min, and for spray inoculation 50 µL of inocula was sprayed on each leaf using a gas chromatography sample syringe connected to a nitrogen gas supply at 2 psi. Inoculated leaves were placed in a biosafety cabinet for 2 h to dry. After that, the leaves were incubated at 4°C for 22 h to allow to *E. coli* O157:H7 cells to attach to the leaf surfaces (LANG *et al.*, 2004), and samples were stored at 10 or 25°C for 7 d.

Microbiological analysis

The inoculated leaves were analyzed at 0.5, 1, 3, and 7 d after storage at 10 or 25°C. The leaves were transferred to a sterile stomacher bag, treated in a stomacher (Easy Mix, AES Laboratoire, France) for 2 min, serially diluted in 0.1 % peptone water and plated on Sorbitol MacConkey Agar supplemented with 0.05 mg/L cefixime and 2.5 mg/L potassium tellurite (CT SMAC). The solidified CT SMAC (20 ml) had been overlaid with 10 ml TSA (thin agar layer format) to facilitate the growth of injured cells. Inoculated plates were incubated at 37°C for 18-24 h.

Statistical analysis

Data presented are means of three experiments with two replicates for each experiment (n=6). Values were analyzed by SPSS software, version 19 (IBM Inc., Armonk, NY) using a univariate general linear model. Differences were considered significant at $p \leq 0.05$.

RESULTS

Behaviour of unstressed *E. coli* O157:H7 recovered from leaf surfaces inoculated by spot, dip and spray methods

The initial number of *E. coli* O157:H7 applied to each leaf was ~ 7.0 log CFU/leaf by each of the three methods. However, significantly higher numbers of *E. coli* O157:H7 cells were recovered from the dip-inoculated rocket leaves (7.10 log CFU/leaf) compared to the spot- or spray-inoculated leaves (6.35-6.71 log CFU/leaf). Further, *E. coli* O157:H7 numbers recovered from the dip-inoculated leaves significantly increased and by 7 d reached 8.20 or 8.37 log CFU/leaf at 10 or 25°C, respectively. The spray and spot methods did not perform differently from each other, and numbers of the pathogen present on spray- and spot-inoculated leaves also increased during storage; however, changes (0.07-0.4 log CFU/leaf) were significantly smaller than with dip-inoculated samples (1.1-1.3 log CFU/leaf) (Table 1).

Table 1 - Viable count of unstressed *E. coli* O157:H7 cells on the surface of rocket leaves stored at 10° or 25°C after inoculation by three methods.

	Inoculation Method					
	10°C			25°C		
Day	Dip	Spot	Spray	Dip	Spot	Spray
0	7.10±0.75aB	6.71±0.41abAB	6.35±0.51aA	7.10±0.75aB	6.71±0.41abAB	6.35±0.51aA
0.5	7.95±0.08bB	6.74±0.52abA	6.43±0.21aA	7.10±0.44aC	6.71±0.32aB	6.24±0.29aA
1	7.98±0.08bC	7.19±0.58aB	6.43±0.20aA	7.67±0.59aB	7.21±0.62aA	6.35±0.76aA
3	7.92±0.85bB	6.50±0.74bA	6.59±0.83aA	7.72±0.08bB	6.81±0.61aA	6.73±0.46aA
7	8.20±0.44bB	6.78±0.48abA	6.72±0.63aA	8.37±0.11bB	6.89±0.46aA	6.78±0.54aA

Values in the same row at each temperature with the same uppercase letter are not significantly different ($p \geq 0.05$).
Values in the same column with the same lowercase letter are not significantly different ($p \geq 0.05$).

Behaviour of acid-stressed *E. coli* O157:H7 recovered from leaf surfaces inoculated by spot, dip and spray methods

The numbers of acid-stressed *E. coli* O157:H7 recovered from rocket leaves differed with the three methods and were ranked in the order of dip > spray > spot inoculation, although the numbers recovered following all inoculation methods were similar ($p > 0.05$). During storage, *E. coli* O157:H7 numbers recovered from dip-, spot- or spray-inoculated rocket leaves significantly increased, and increases by 7 d were 0.9 to 1.3 log CFU/leaf at 10°C and 1.2 to 1.6 log CFU/leaf at 25°C. Meanwhile, the highest *E coli* O157:H7 numbers present by 7 d storage at both temperatures were on rocket leaves inoculated by dipping (8.51-8.78 log CFU /leaf) and by spraying (7.79-7.93 log CFU /leaf) (Table 2).

Behaviour of cold-stressed *E. coli* O157:H7 recovered from leaf surfaces inoculated by spot, dip and spray methods

E. coli O157:H7 numbers recovered from the dip-inoculated leaves were significantly higher (7.53 log CFU/leaf) than those recovered from either the spot-inoculated (6.65 log CFU/leaf) or spray-inoculated leaves (6.48 log CFU/leaf). During storage, *E. coli* O157:H7 numbers recovered from rocket leaves, regardless of the inoculation method used, significantly increased (0.8-1.29 log CFU/leaf) at 10 and 25°C by 7 d (Table 3).

Behaviour of starvation-stressed *E. coli* O157:H7 recovered from leaf surfaces inoculated by spot, dip and spray methods

As with cold-stressed cells, numbers of starvation-stressed *E. coli* O157:H7 recovered from the dip-inoculated rocket leaves were significantly higher (7.50 log CFU/leaf) than those recovered from the spot- (6.64 log CFU/leaf) or spray-inoculated leaves (6.46 log CFU/leaf). During storage at 10 or 25°C, the numbers of *E. coli* O157:H7 cells recovered from rocket leaves inoculated using the three methods remained constant for 1 day, but after that there was a significant increase in their numbers (0.7-1.1 log CFU/leaf). Spot and spray inoculation methods had the same effect on *E. coli* O157:H7 numbers during storage; however dip inoculation enabled higher recoveries from the leaves at all storage intervals (Table 4).

Table 2 - Viable count of acid-stressed *E. coli* O157:H7 cells on the surface of rocket leaves stored at 10° or 25°C after inoculation by three methods.

	Inoculation Method					
	10°C			25°C		
Day	Dip	Spot	Spray	Dip	Spot	Spray
0	7.23±0.47aA	6.49±0.75aA	6.75±0.84aA	7.23±0.47aA	6.49±0.75aA	6.75±0.84aA
0.5	7.81±0.39bB	6.76±0.26aA	6.89±0.51aA	8.09±0.34aB	7.12±0.24aA	7.16±0.58aA
1	8.07±0.12bC	6.94±0.72abA	7.54±0.51bB	8.27±0.36abB	7.47±0.13abA	7.59±0.16abA
3	8.12±0.08bB	7.42±0.41bA	7.66±0.49bAB	8.50±0.18bcC	7.49±0.24abA	7.82±0.2bB
7	8.51±0.04cC	7.36±0.25bA	7.79±0.11bB	8.87±0.07cC	7.71±0.07bA	7.93±0.04bB

Values in the same row at each temperature with the same uppercase letter are not significantly different ($p \geq 0.05$).
Values in the same column with the same lowercase letter are not significantly different ($p \geq 0.05$).

Table 3 - Viable count of cold-stressed *E. coli* O157:H7 cells on the surface of rocket leaves stored at 10° or 25°C after inoculation by three methods.

	Inoculation Method					
	10°C			25°C		
Day	Dip	Spot	Spray	Dip	Spot	Spray
0	7.53±0.16aB	6.65±0.32aA	6.48±0.25aA	7.53±0.16aB	6.65±0.32aA	6.48±0.25aA
0.5	7.99±0.12bB	7.24±0.13bA	7.01±0.29bcA	7.53±0.51aB	6.90±0.57aA	6.76±0.48aA
1	7.81±0.68abB	7.23±0.15bA	6.69±0.66abA	7.86±0.10bB	7.06±0.57abA	6.97±0.51abA
3	8.15±0.06bcB	7.39±0.04bcA	7.34±0.03cA	8.35±0.04bC	7.52±0.06bcB	7.41±0.09bcA
7	8.43±0.09cB	7.45±0.08cA	7.38±0.09cA	8.39±0.05bC	7.94±0.07cB	7.62±0.07cA

Values in the same row at each temperature with the same uppercase letter are not significantly different ($p \geq 0.05$).
Values in the same column with the same lowercase letter are not significantly different ($p \geq 0.05$).

Behaviour of salt-stressed *E. coli* O157:H7 recovered from leaf surfaces inoculated by spot, dip and spray methods

Numbers of salt-stressed *E. coli* O157:H7 were also higher ($p < 0.05$) on dip-inoculated leaves than those of other treatments. Their numbers significantly increased on spray- or dip-inoculated leaves, and by 7 d reached 7.31 and 8.44 log CFU/leaf, respectively, at 10°C and 7.45 and 8.32 log CFU/leaf, respectively, at 25°C. However, there was no change in the numbers of *E. coli* O157:H7 recovered from spot-inoculated rocket leaves ($p > 0.05$). Increases during storage were 0.4 log CFU/leaf on samples spot-inoculated, but numbers on leaves from the other treatments increased 1.0 - 1.3 log CFU/leaf at both temperatures (Table 5).

Table 4 - Viable count of starvation-stressed *E. coli* O157:H7 cells on the surface of rocket leaves stored at 10° or 25°C after inoculation by three methods.

	Inoculation Method					
	10°C			25°C		
Day	Dip	Spot	Spray	Dip	Spot	Spray
0	7.50±0.19aB	6.64±0.30aA	6.46±0.27aA	7.50±0.19aB	6.64±.30aA	6.46±0.27aA
0.5	7.50±0.47aB	6.74±0.56aA	6.77±0.73abA	7.86±0.52aB	7.01±0.59aA	6.79±0.50aA
1	7.81±0.38aB	6.96±0.53abA	6.79±0.50abA	8.11±0.47abB	7.08±0.39aA	6.93±0.51aA
3	8.14±0.06bC	7.43±0.05cB	7.31±0.07bA	8.32±0.11bcB	7.54±0.07bA	7.44±.0.06bA
7	8.46±0.12bB	7.36±0.06bcA	7.21±0.43bA	8.51±0.06cB	7.54±0.07bA	7.54±0.08bA

Values in the same row at each temperature with the same uppercase letter are not significantly different ($p \geq 0.05$).
Values in the same column with the same lowercase letter are not significantly different ($p \geq 0.05$).

Table 5 - Viable count of salt-stressed *E. coli* O157:H7 cells on the surface of rocket leaves stored at 10° or 25°C after inoculation by three methods.

	Inoculation Method					
	10°C			25°C		
Day	Dip	Spot	Spray	Dip	Spot	Spray
0	7.10±0.91aA	6.94±0.38aA	6.45±0.26aA	7.10±0.81aA	6.94±0.38aA	6.45±0.26aA
0.5	7.81±0.37bB	6.86±0.49aA	6.82±0.55abA	7.86±0.52aB	6.99±0.60aA	6.81±0.52aA
1	7.86±0.38bcB	6.88±0.53aA	6.88±0.50abA	8.08±0.49aB	7.09±0.57aA	6.97±0.50aA
3	7.89±0.40bcB	7.02±0.50aA	6.94±0.52abA	8.13±0.51aB	7.20±0.53aA	7.30±0.52aA
7	8.44±0.07cB	7.36±0.15aA	7.31±0.17bA	8.32±0.11bB	7.38±0.44aA	7.45±0.06bA

Values in the same row at each temperature with the same uppercase letter are not significantly different ($p \geq 0.05$).
Values in the same column with the same lowercase letter are not significantly different ($p \geq 0.05$).

DISCUSSION

Different inoculation methods have been used experimentally to contaminate fresh produce in studies of the survival or inactivation of pathogens (AL-NABULSI *et al.*, 2014; LANG *et al.*, 2004 a,b; SINGH *et al.*, 2002). However, it is possible that the method chosen for inoculation may affect pathogen behaviours (survival, growth, injury, or death). In the present study three inoculation techniques (dip, spot and spray) were used and there was variability in the number of *E. coli* O157:H7 present on the leaves contaminated. It was found that dipping yielded larger numbers of unstressed or stressed *E. coli* O157:H7 cells on rocket leaves. This may have been because of the greater exposure of leaf surfaces, including cut surfaces where some cells could have been internalized. These results are similar to those obtained by LANG *et al.* (2004a) who showed that higher numbers of *E. coli* O157:H7 and *Salmonella* were recovered from dip-inoculated tomatoes compared to those spot- or spray–inoculated. In another study, LANG *et al.* (2004b) observed that applying the cell suspension to the surface of lettuce by dipping enhanced the internalization of bacteria at the cut edge and via stomata which can facilitate bacterial access to internal leaf tissue. The results of the current study also indicated that bacterial numbers recovered from spot-inoculated leaves were not significantly different from those recovered from those that were spray-inoculated. Similarly, LANG *et al.* (2004b) found that the number of *E. coli* O157:H7 and *Salmonella* recovered from lettuce when inoculated by spot or spray methods were similar. However, they recommended using the spot method as the standard for inoculation in studying the efficacy of sanitizers against pathogenic bacteria. SINGH *et al.* (2002) found that some sanitizers (thyme oil, aqueous chlorine dioxide, ozonated water) were less effective against *E. coli* O157:H7 on lettuce inoculated by dipping or sprinkling than by the spot or drop method. It should be noted that even when fresh produce was washed and sanitized using chemical agents such as chlorine, only a 1-2 log microbial reduction was achieved (OLAIMAT and HOLLEY, 2012).

Unstressed *E. coli* O157:H7 cells were able to grow when inoculated by dipping at 10 or 25°C, but cells inoculated by spraying or spotting survived without significant change in numbers at both temperatures over 7 d storage. These results are similar to those reported by CHANG and FANG (2007) who found that *E. coli* O157:H7 numbers on lettuce increased by 2.7 log CFU/g at 22°C, although they decreased by 1.4 log CFU/g at 4°C. FRANCIS and O'BEIRNE (2001) also reported that *E. coli* O157:H7 numbers increased by up to 2.5 log CFU/g after 12 d on spot-inoculated shredded lettuce, coleslaw and soybean sprouts at 8°C. LUO *et al.* (2010) found that storage of spray-inoculated lettuce at 5°C allowed *E. coli* O157:H7 to survive, but its growth was limited. At 12°C there was more than a 2.0 log CFU/g increase in *E. coli* O157:H7 numbers after 3 d storage. In contrast, MARKLAND *et al.* (2013) did not detect *E. coli* O157:H7 cells after 4 d on basil plants that were spray irrigated.

The behaviour of microorganisms in food products depends on the interaction of intrinsic and extrinsic factors such as temperature, pH, and water activity. Bacteria may encounter sub-lethal stresses in variety of food products, particularly minimally processed food such as fresh produce. Responses of bacteria to these stresses may enhance their survival under more severe conditions, enhance their resistance to subsequent processing conditions and perhaps enhance virulence. Thus, understanding the effects of environmental stress on the behaviour of pathogens is important in order to assess and minimize the risk of foodborne illness (CHUNG *et al.*, 2006). In *E. coli* O157:H7 exposure to stress can initiate several mechanisms to minimize the effects of the challenge. For example, the *rpoS* gene regulates expression of > 50 proteins that are involved in the general stress response. Also, heat and cold shock genes can play a major role in the level of expression of the response by stressed *E. coli* O157:H7. These mechanisms facilitate adaptation of *E. coli* O157:H7 to environmental change and increase its survival (CHAURET 2011). In the current study, numbers of stressed *E. coli* O157:H7 recovered by the three different methods increased significantly at 10° and 25°C. In contrast, MCEVOY *et al.* (2009) found that the behaviour of cold-stressed *E. coli* O157:H7 was similar to that of unstressed cells on fresh iceberg lettuce where the cold-stressed and unstressed cells grew significantly at 30°C, but survived without changes in their numbers at the non-permissive growth temperature of 5°C after 8 d. Several factors are likely to affect growth and survival of *E. coli* O157:H7 on fresh produce including its type (pH, surface smoothness, porosity, nutrient availability), physiological state, moisture, storage temperature > 7°C, and the identity of the bacterial strain. It was of interest from the present work that stressed cells of a 4 strain *E. coli* O157:H7 cocktail on Rocket leaves were able to increase in numbers during a week of storage at 10° and 25°C to similar extents, but unstressed cells did not.

In conclusion, it appears that the method used for bacterial inoculation of produce leaves can influence the levels of *E. coli* O157:H7 recovered from treated samples. The greatest uptake of cells from the inoculum occurred when leaves were dipped. Thus the importance of controlling the quality of water used in produce plant flumes and for produce immersion becomes apparent. Spot and spray inoculation yielded lower but similar levels of contamination. Thus produce contact with unclean equipment surfaces,

handling of produce in an unsanitary manner by employees and the occurrence of aerosols during processing can increase the bacterial burden that is likely to occur on the final product. Most importantly, when cells stressed by acidic pH, cold, starvation or salt exposure were inoculated on Rocket leaves, cells were able to grow slowly at both 10° and 25°C, whereas unstressed cells did not increase in numbers during 7 d storage. This unanticipated feature of *E. coli* O157:H7 may enhance its ability to be spread through shipments of produce during distribution, increasing risks associated with this foodborne pathogen.

ACKNOWLEDGMENTS

The authors thank the Deanship of Scientific Research at Jordan University of Science and Technology (grant 282/2011).

REFERENCES

Al-Nabulsi A.A., Osaili T.M., Obaidat H.M., Shaker R.R., Awaisheh S.S. and Holley R.A. 2014. Inactivation of stressed *Escherichia coli* O157:H7 cells on the surfaces of rocket salad leaves by chlorine and peroxyacetic acid. J. Food Prot. 77: 32-39.

Beuchat L.R., Farber J.M., Garrett M., Harris L.J., Parsih M.E., Suslow T.V. and Busta F.F. 2003. Standardization of a method to determine the efficacy of sanitizers in inactivating human pathogenic microorganisms on raw fruits and vegetables. Compr. Rev. Food Sci. Food Safety 6: 174-178.

Brandl M.T. 2006. Fitness of human enteric pathogens on plants and implications for food safety. Ann. Rev. Phytopathol. 44: 367-392.

Chang J.M. and Fang T.J. 2007. Survival of *Escherichia coli* O157:H7 and *Salmonella enterica* serovars Typhimurium in iceberg lettuce and the antimicrobial effect of rice vinegar against *E. coli* O157:H7. Food Microbiol. 24: 745-751.

Chauret C. 2011. Survival and control of *Escherichia coli* O157:H7 in foods, beverages, soil and water. Virulence 2: 593-601.

Chung H.J., Bang W. and Drake M.A. 2006. Stress response of *Escherichia coli*. Compr. Rev. Food Sci. Food Safety 5: 52-64.

Cooley M.B., Miller W.G. and Mandrell R.E. 2003. Colonization of *Arabidopsis thaliana* with *Salmonella enterica* and enterohemorrhagic *Escherichia coli* O157:H7 and competition by *Enterobacter asburiae*. Appl. Environ. Microbiol. 69: 4915-4926.

Delaquis P., Bach S. and Dinu L. 2007. Behavior of *Escherichia coli* O157:H7 in leafy vegetables. J. Food Prot. 70: 1966-1974.

Dinu L.D., Delaquis P. and Bach S. 2009. Nonculturable response of animal enteropathogens in the agricultural environment and implications for food safety. J. Food Prot. 72: 1342-1354.

Francis G.A. and O'Beirne O. 2001. Effects of vegetable type, package atmosphere and storage temperature on growth and survival of *Escherichia coli* O157:H7 and *Listeria monocytogenes*. J. Ind. Microbiol. Biotech. 27: 111-116.

Hajmeer M., Ceylan E., Marsden J.L. and Fung D.Y.C. 2006. Impact of sodium chloride on *Escherichia coli* O157:H7 and *Staphylococcus aureus* analysed using transmission electron microscopy. Food Microbiol. 23: 446-452.

Islam M., Doyle M.P., Phatak S.C., Millner P. and Jiang X.P. 2004. Persistence of enterohemorrhagic *Escherichia coli* O157:H7 in soil and on leaf lettuce and parsley grown in fields treated with contaminated manure composts or irrigation water. J. Food Prot. 67: 1365-1370.

Lang M.M., Harris L.J. and Beuchat L.R. 2004a. Evaluation of inoculation method and inoculum drying time for their effects on survival and efficiency of recovery of *Escherichia coli* O157:H7, *Salmonella*, and *Listeria monocytogenes* inoculated on the surface of tomatoes. J. Food Prot. 67: 732-741.

Lang M.M., Harris L.J. and Beuchat L.R. 2004b. Survival and recovery of *Escherichia coli* O157:H7, *Salmonella*, and *Listeria monocytogenes* on lettuce and parsley as affected by method of inoculation, time between inoculation and analysis, and treatment with chlorinated water. J. Food Prot. 67: 1092-1103.

Leenanon B. and Drake M.A. 2001. Acid stress, starvation, and cold stress affect poststress behavior of *Escherichia coli* O157:H7 and nonpathogenic *Escherichia coli*. J. Food Prot. 64: 970-974.

Luo Y., He Q. and McEvoy J.L. 2010. Effect of storage temperature and duration on the behavior of *Escherichia coli* O157:H7 on packaged fresh-cut salad containing Romaine and iceberg lettuce. J. Food Sci. 75: M390-M397.

Luo Y., He Q., McEvoy J.L. and Conway W.S. 2009. Fate of *Escherichia coli* O157:H7 in the presence of indigenous microorganisms on commercially packaged baby spinach as impacted by storage temperature and time. J. Food Prot. 72: 2038-45.

Markland S.M., Shortlidge K.L., Hoover D.G., Yaron S., Patel J., Singh A., Sharma M. and Kniel K.E. 2013. Survival of pathogenic *Escherichia coli* on basil, lettuce, and spinach. Zoonoses Public Health 60: 563-71.

McEvoy J.L., Luo Y., Zhou B., Feng H. and Conway W.S. 2009. Potential of *Escherichia coli* O157:H7 to grow on field-cored lettuce as impacted by postharvest storage time and temperature. Int. J. Food Microbiol. 128: 506-509.

Nygard K., Lassen J., Vold L., Andersson Y., Fisher I. and Lofdahl S. 2008. Outbreak of *Salmonella* Thompson infections linked to imported rucola lettuce. Foodborne Pathog. Dis. 5: 165-173.

Olaimat A.N. and Holley R.A. 2012. Factors influencing the microbial safety of fresh produce: A review. Food Microbiol. 32: 1-19.

Painter J.A., Hoekstra R.M., Ayers T., Tauxe R.V., Braden C.R., Angulo F.J. and Griffin P.M. 2013. Attribution of foodborne illnesses, hospitalizations, and deaths to food commodities by using outbreak data, United States, 1998-2008. Emerg. Infect. Dis. 19: 407-415.

Scallan E., Hoekstra R.M., Angulo F.J., Tauxe R.V., Widdowson M.A., Roy S.L., Jones J.L. and Griffin P.M.. 2011. Foodborne illness acquired in the United States— major pathogens. Emerg. Infect. Dis. 17: 7-15.

Singh N., Singh R.K., Bhunia A.K. and Stroshine R.L. 2002. Efficacy of chlorine dioxide, ozone, and thyme essential oil or a sequential washing in killing *Escherichia coli* O157:H7 on lettuce and baby carrots. LWT Food Sci. Techn. 35: 720-729.

Thomas M.K., Murray R., Flockhart L., Pintar K., Pollari F., Fazil A., Nesbitt A. and Marshall B. 2013. Estimates of the burden of foodborne illness in Canada for 30 specified pathogens and unspecified agents, circa 2006. Foodborne Pathog. Dis. 10: 639-648.

World Health Organization (WHO). 2011. Enterohaemorrhagic *Escherichia coli* (EHEC), Fact sheet N°125. Available at: http://www.who.int/mediacentre/factsheets/fs125/en/ (Accessed on May 30, 2014).

Paper Received June 23, 2014 Accepted April 15, 2015