International Journal of Food Microbiology 128 (2009) 506–509

Contents lists available at ScienceDirect

International Journal of Food Microbiology

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

Short communication

Potential of *Escherichia coli* O157:H7 to grow on field-cored lettuce as impacted by postharvest storage time and temperature

James L. McEvoy [a,*], Yaguang Luo [a], William Conway [a], Bin Zhou [b], Hao Feng [b]

[a] Produce Quality and Safety Laboratory, U.S. Department of Agriculture, Agricultural Research Service, Building 002, Room 117, 10300 Baltimore Avenue, Beltsville, MD 20705, United States
[b] Department of Food Science and Human Nutrition, University of Illinois at Urbana-Champaign, Urbana, IL 61801, United States

ARTICLE INFO

Article history:
Received 6 February 2008
Received in revised form 19 June 2008
Accepted 19 August 2008

Keywords:
E. coli O157:H7
Lettuce
Field coring
Food safety

ABSTRACT

A recent development in iceberg lettuce harvesting is field coring, the technique of removing the outer leaves and the cores of the lettuce heads at the time of harvesting in order to reduce shipping waste and maximize production yield. However, this method may increase the potential for contamination during field procedures and therefore, it is important to evaluate the survival and growth of *Escherichia coli* O157:H7 on pre-cored lettuce under simulated field conditions. Using a coring knife artificially contaminated with 2×10^5 cells of *E. coli* O157:H7, the transfer of the pathogen to lettuce heads and subsequent growth of the pathogen at simulated field and refrigerated temperatures (30 and 5 °C) were examined. No significant ($P>0.05$) growth or loss of viability of *E. coli* O157:H7 was noted at 5 °C during an 8 h incubation period. However, at 30 °C, significant ($P<0.001$) increases in *E. coli* O157:H7 populations occurred between 0 to 4 h and 4 to 8 h. Regardless of whether *E. coli* O157:H7 were cold-stressed prior to use as inoculum, *E.coli* O157:H7 populations increased by more than 2.0 log cfu/g at 30 °C from 0 to 8 h. A single contaminated coring knife was found to successively inoculate at least nineteen lettuce heads. These findings suggest that preventing contamination of the coring knife and cored lettuce, as well as prompt chilling of freshly cored lettuce heads, are necessary steps to ensure the safety of field-cored iceberg lettuce.

1. Introduction

Iceberg lettuce is a major ingredient of many packaged fresh-cut salads. The consumption of lettuce has grown rapidly over the past decade, as has the fresh-cut industry; and these trends are expected to continue. Iceberg lettuce is traditionally harvested with the cores intact, which are later removed during in-plant trimming along with additional outer leaves before processing. Field coring and trimming of iceberg lettuce is a new industry harvesting practice, which involves removal of the core and dirty or damaged wrapper leaves of lettuce in the field in order to increase processing plant production yields from the traditional levels of 60–70% to nearly 100% (Anonymous, 1996). After coring, lettuce heads are often sprayed with a chlorine solution and collected in field holding bins. The harvested lettuce is then transported to a cooling facility and vacuum cooled to below 5 °C, usually within 4 h after coring. However, cooling can be delayed for several additional hours due to the distance from field to cooling facility, or if the load of the lettuce requiring cooling exceeds the capacity of the cooling equipment.

Several recent outbreaks of *Escherichia coli* O157:H7 associated with lettuce consumption have been reported (Mermin et al., 1996; Ackers et al., 1998; Hilborn et al., 1999; Beuchat 2002; Harris et al., 2003; Delaquis et al., 2007). At least one of these cases involved lettuce contaminated postharvest (Hilborn et al., 1999). In this incident, harvested mesclun lettuce may have come into contact with the pathogen via cross-contamination from processing wash water. *E. coli* O157:H7 can also become associated with produce in the field via contaminated irrigation water (Wachtel et al., 2001, 2002; Solomon et al., 2003). Although there is little published data regarding the growth of human pathogens on lettuce during postharvest handling, Wachtel et al. (2003), and Wachtel and Charkowski (2002) demonstrated that processed lettuce, once contaminated, can serve as a good substrate for growth and/or survival of *E. coli* O157:H7. Tissue damage, either from mechanical damage or enzymatic damage, may enhance the growth of human pathogens on produce. Wells and Butterfield (1997) found that potato, carrot, and pepper disks damaged by the action of the soft rot bacterium *Erwinia carotovora* enhanced the growth of *Salmonella typhimurium* compared to disks inoculated with *Salmonella* alone. In another study (Gleeson and O'Beirne, 2005) it was determined that cutting with a dull knife or tearing of vegetable tissues allows for better growth of *E. coli* than clean cutting with a sharp knife. Seo and Frank (1999) observed that *E. coli* O157:H7 cells attached preferentially to damaged tissues of cut edges over intact surface tissues. The purpose of this study was to determine the potential for transfer of *E.coli* O157:H7 from a

* Corresponding author. Tel.: +1 301 504 6983; fax: +1 301 504 5107.
E-mail address: james.mcevoy@ars.usda.gov (J.L. McEvoy).

0168-1605/$ – see front matter © 2008 Published by Elsevier B.V.
doi:10.1016/j.ijfoodmicro.2008.08.008

contaminated coring knife to the cored lettuce, and to investigate the effect of time and temperature post-coring on the survival and growth of *E.coli* O157:H7 on lettuce.

2. Materials and methods

2.1. Bacterial strains and growth conditions

A nalidixic acid resistant derivative of *E. coli* O157:H7 strain F6460, isolated from patient fecal samples during a 1999 Nebraska lettuce outbreak, was used throughout this study. The cultures were stored at −80 °C in Luria-Bertani (LB) broth (Difco Laboratories, Detroit, MI, USA) containing 25% (vol/vol) glycerol. To prepare the inoculum, *E. coli* O157:H7 was grown overnight on LB broth at 37 °C. The inoculum was sampled before and after lettuce inoculation to quantify the pathogen.

The cold-stressed *E. coli* O157:H7 were prepared by growing the culture overnight in LB_{Nal} broth and then placing it at 5 °C for 24 h prior to diluting in PBS for use as inoculum.

2.2. Lettuce contamination

Fresh iceberg lettuce was obtained from a local produce wholesale market in Jessup, MD, USA. Lettuce heads were pre-selected for similar shape and size. Soiled and damaged outer lettuce leaves were removed and the stem end was trimmed. Lettuce coring knives (Agricultural Supplies Inc., Salinas, CA, USA) were sanitized with 70% ethanol prior to each use. The outer edge of the coring ring (about 0.5 cm from cutting edge) was inoculated evenly with 20 µl (2×10^5 cells) of *E. coli* O157:H7. The inoculum was allowed to partially air dry on the coring knife for ~30 s prior to coring of the lettuce heads. PBS solution (20 µl) was used for the negative controls. Each lettuce head was cored with this artificially contaminated coring knife. There were five replications for each sampling time and temperature and the experiment was performed twice with an overnight culture and once with a stressed culture of *E. coli* O157:H7. The heads were placed in gas permeable plastic bags and stored at 5 and 30 °C for 0, 4 and 8 h. At each sampling time, contaminated lettuce was harvested by excising approximately 25 g of tissue from the inside edge of the cored area (about 0.5 cm deep from the original edge) with a sterile knife. The samples were homogenized in a Stomacher 400 Biomaster with 150 ml PBS (Seward Limited, London, England) for 2 min at 230 RPM. Homogenates were filtered through sterile filter stomach bags, and duplicate samples were plated onto LB_{Nal} agar plates using a Wasp II Spiral Plater (DW Scientific, West Yorkshire, England). Bacterial colonies were incubated overnight LB_{Nal} at 37 °C and enumerated with an automated plate counter (Protoc, Synoptics, Cambridge, England). Colonies grown on LB_{Nal} media were confirmed to be *E.coli* O157:H7 by RIM™ *E. coli* O157:H7 Latex Test (REMEL Inc., Lenexa, KS). Enumeration of the total aerobic bacterial counts and yeast and mold counts was accomplished by plating the filtrates or their appropriate dilutions onto tryptic soy agar and potato dextrose agar supplemented with chloramphenicol and incubated at 28 °C for 2 days and 25 °C for 3 days, respectively.

2.3. Transfer of E. coli O157:H7 from a contaminated coring knife to successive lettuce heads

The coring knife was artificially contaminated as described above with approximately 5×10^5 *E. coli* O157:H7 cells. The knife was then used to successively core 20 lettuce heads. The cored heads were either sampled immediately or placed in plastic boxes at 30 °C for 4 h prior to sampling. Harvesting of lettuce tissue and enumeration of *E.coli* O157:H7 cells were as described above for the first ten lettuce heads. For cored lettuce heads from 11 to 20, the 25 g of harvested tissue was homogenized in 50 ml tryptic soy broth (TSB) with a Stomacher 400 Biomaster as described above. Homogenates were incubated at 37 °C for 24 h for *E.coli* O157:H7 enrichment. Dilutions of the enrichment samples were plated on LB_{Nal} and examined for bacterial growth following 18 h of incubation at 37 °C. The experiment was repeated three times.

2.4. Experimental design and statistical analyses

The experiment was conducted using a completely randomized design. Data were analyzed as a general linear model using the PROC MIXED procedure (SAS Institute Inc., 1999) with storage time and temperature as the main factors. The assumptions of the linear model were checked. The variance grouping technique was used to correct variance heterogeneity for the means comparisons. Mean comparisons were done with Sidak adjusted *p*-values so that the experiment-wise error was ≤0.05.

3. Results and discussion

A coring knife harboring 2×10^5 cells of *E. coli* O157:H7 on the outside of its cutting ring transferred measurable amounts of bacteria to the cut surface of cored iceberg lettuce heads (Fig. 1). Immediately after coring, greater than 3.0 log cfu/g *E. coli* O157:H7 were recovered from the cored area. After 4 and 8 h incubation at 5 °C, no significant ($P>0.05$) differences were noted in the pathogen populations (Fig. 1A), demonstrating the effectiveness of refrigerated temperatures at preventing growth of *E. coli* O157:H7. However, at 30 °C, *E. coli* O157:H7 populations increased significantly ($P<0.001$) after 4 h, followed

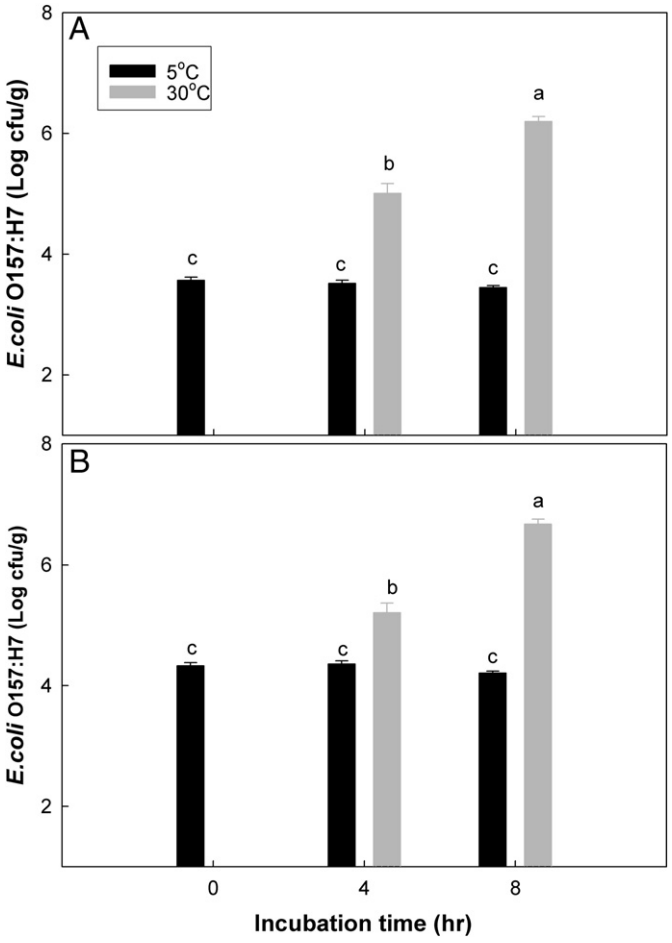

Fig. 1. The effect of postharvest holding time and temperature on the growth of *E. coli* O157:H7 on simulated field-cored lettuce. Vertical bars are the means of ten replications ± S.E. Panel A — lettuce inoculated with *E.coli* O157:H7 from an overnight culture; Panel B — lettuce inoculated with *E.coli* O157:H7 that was cold-stressed.

by an additional increase after 8 h. The *E. coli* O157:H7 inoculation level of 2×10^5 cells may be higher than one would expect to encounter on the cutting surface of a coring knife. This inoculum level ensured reliable detection of the organism in the samples taken immediately after inoculation.

E.coli O157:H7 that exists in the environment outside of animal hosts may encounter cold and nutrient stress (Delaquis et al., 2007). In order to mimic this situation, an additional experiment was conducted using *E. coli* O157:H7 that had been stressed by storage of a stationary LB culture for 24 h at 4 °C. Fig. 1B indicates that stressed *E. coli* O157:H7 behaved similarly to non-stressed cells with respect to its growth on freshly cored iceberg lettuce. No significant ($P>0.05$) differences were noted among the 5 °C samples in the number of recovered cells between any time points, while among 30 °C samples, significant ($P<0.001$) differences were seen between 0 and 4 h and 4 and 8 h. It is unknown whether *E. coli* O157:H7 cells that are cold-stressed for longer than 24 h or exposed to other forms of stress (nutrient, heat, chemical exposure, etc.) would behave in a similar manner. Furthermore, other *E. coli* O157:H7 isolates may behave differently with respect to cold stress and subsequent growth on cored lettuce.

Results similar to those seen with *E. coli* O157:H7 were obtained for total aerobic mesophilic bacteria (Fig. 2A). These numbers, which included the artificially inoculated *E. coli* O157:H7, were approximately 1.0 log cfu/g higher than the *E. coli* O157:H7 numbers immediately after coring and remained essentially unchanged during the 8 h sampling time span for the 5 °C incubated samples. Similar to the *E. coli* O157:H7 populations, the total aerobic counts increased significantly from 0 to 4 h and from 4 to 8 h post-coring when samples were incubated at 30 °C. Counts of yeasts and molds were more variable than the *E. coli* or total aerobic counts but followed the same trend (Fig. 2B). Initial counts were about 2.7 log cfu/g and remained fairly constant at 5 °C for up to 8 h. At 30 °C yeasts and molds increased about 0.5 and 1.0 log cfu/g at 4 and 8 h, respectively. The increase in total mesophilic bacteria and yeasts and molds at the elevated temperature, even after as little as 4 h incubation, could impact the quality of the lettuce during subsequent storage dependent upon the makeup of the unknown background microflora. Furthermore, these data indicate that the holding temperature of field-cored lettuce influenced the growth of *E.coli* O157:H7 and the natural microflora; also, *E. coli* O157:H7 grew well in the presence of such organisms.

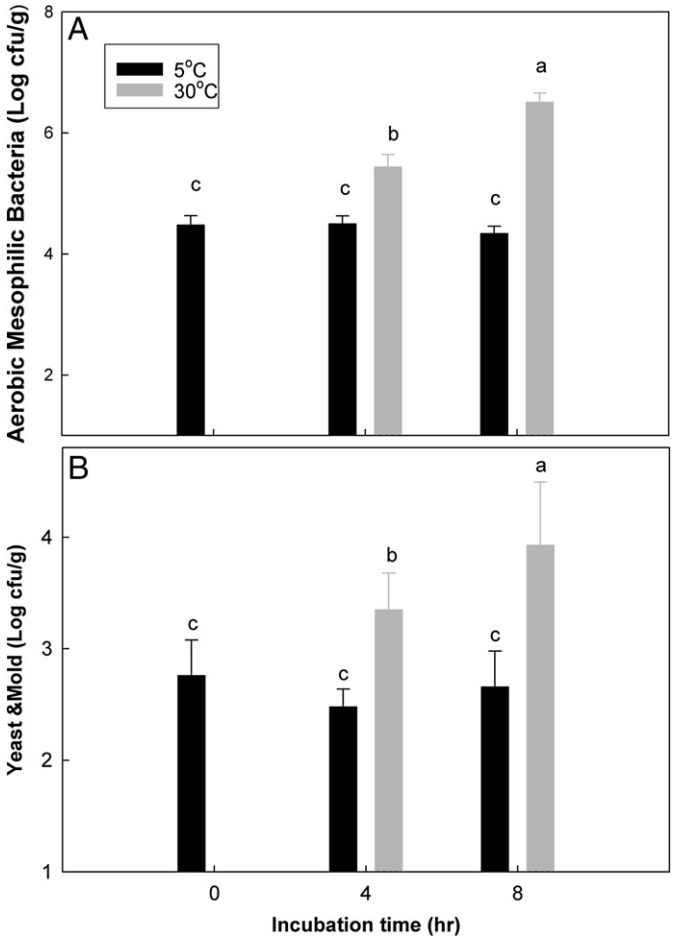

Fig. 2. The effect of postharvest holding time and temperature on total aerobic mesophilic bacteria (A) and yeasts and molds (B) on simulated field-cored lettuce. Vertical bars are the means of five replications ± S.E.

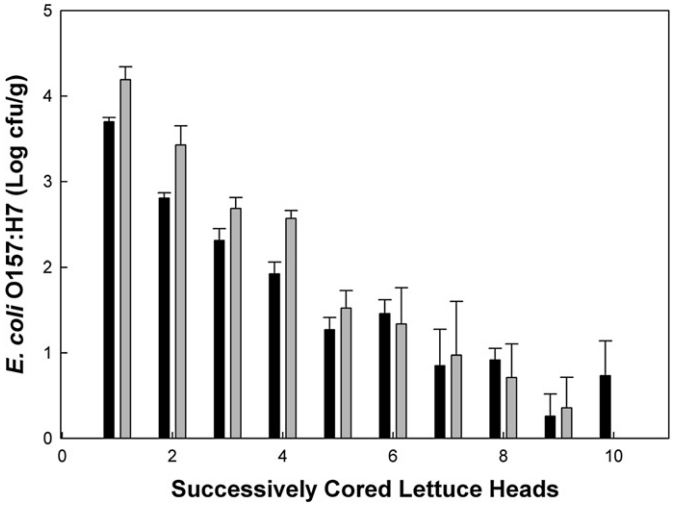

Fig. 3. Transfer of *E. coli* O157:H7 to simulated field-cored lettuce via a contaminated coring knife. Samples were either harvested immediately after coring or after incubation at 30 °C for 4 h. Vertical bars are the means of three replications ± S.E.

Frequent sanitation of the coring knives during use should be a priority of lettuce harvesters since *E. coli* O157:H7 can easily be transferred from a knife to successive heads of iceberg lettuce (Fig. 3). Sodium hypoclorite at 200 ppm was effective at reducing *E. coli* cells on stainless steel surfaces by more than 1 log even after cells were allowed to adhere to the surface for 1 h (Rossoni and Gaylarde, 2000). If the knife is dipped in a chlorine solution frequently during use, adherence should be much less and sanitizing efficacy greater. In two of three replications, *E. coli* O157:H7 was detected on the tenth successively cored lettuce head immediately after coring. A 4 h incubation of the cored heads at 30 °C generally resulted in higher *E. coli* O157:H7 populations. All samples through the sixth successively cored head, whether incubated at 30 °C or not, contained *E. coli* O157:H7 populations above the detection limit of 5 cells in 25 g of lettuce tissue. Following 24 h enrichment in TSB at 37 °C, *E.coli* O157:H7 was detected up to the 19th lettuce head.

4. Conclusions

Significant increases in *E. coli* O157:H7 occurred at the cored site of iceberg lettuce heads within 4 h at 30 °C. The population of organisms continued to increase to at least 8 h post-coring. Thus to minimize food safety concerns, care should be taken in the field during harvesting and field coring of iceberg lettuce to ensure that chilling of the produce occurs as quickly as possible. This research provides scientific information for the fresh-cut produce industry to make informed decisions regarding the development and implementation of Good Agricultural Practice (GAP) in order to minimize food safety risks associated with lettuce.

References

Ackers, M.L., Mahon, B.E., Leahy, E., Goode, B., Damrow, T., Hayes, P.S., Bibb, W.F., Rice, D.H., Barrett, T.J., Hutwagner, L., Griffin, P.M., Slutsker, L., 1998. An outbreak of *Escherichia coli* O157:H7 infections associated with leaf lettuce consumption. The Journal of Infectious Diseases 177 (6), 1588–1593.

Anonymous, 1996. Spotlight on trimmed and cored lettuce. Fresh Cut 4 (10), 17.

Beuchat, L.R., 2002. Ecological factors influencing survival and growth of human pathogens on raw fruits and vegetables. Microbes and Infection 4 (4), 413–423.

Delaquis, P., Bach, S., Dinu, L.D., 2007. Behavior of *Escherichia coli* O157:H7 in leafy vegetables. Journal of Food Protection 70 (8), 1966–1974.

Gleeson, E., O'Beirne, D., 2005. Effects of process severity on survival and growth of *Escherichia coli* and *Listeria innocua* on minimally processed vegetables. Food Control 16 (8), 677–685.

Harris, L.J., Farber, J.N., Beuchat, L.R., Parish, M.E., Suslow, T.V., Garrett, E.H., Busta, F.F., 2003. Outbreaks associated with fresh produce: incidence, growth, and survival of pathogens in fresh and fresh-cut produce. Comprehensive Reviews. Food Science and Food Safety, vol. 2, pp. 78–141 (Supplement).

Hilborn, E.D., Mermin, J.H., Mshar, P.A., Hadler, J.L., Voetsch, A., Wojtkunski, C., Swartz, M., Mshar, R., Lambert-Fair, M.A., Farrar, J.A., Glynn, M.K., Slutsker, L., 1999. A multistate outbreak of *Escherichia coli* O157:H7 infections associated with consumption of mesclun lettuce. Archives of Internal Medicine 159 (15), 1758–1764.

Mermin, J., Mead, P., Gensheimer, K., Griffin, P., 1996. Outbreak of *Escherichia coli* O157: H7 infections among boy scouts in Maine. p. 257, K44. Abstract 36th Interscience Conference Antimicrobial Agents Chemistry—Other. American Society of Microbiology, Washington D.C.

Rossoni, E.M.M., Gaylarde, C.C., 2000. Comparison of sodium hypochlorite and peracetic acid as sanitizing agents for stainless steel food processing surfaces using epifluorescence microscopy. International Journal of Food Microbiology 61 (1), 81–85.

SAS Institute Inc. 1999. SAS/STAT User's Guide, Version 8. 3884 pp.

Seo, K.H., Frank, J.F., 1999. Attachment of *Escherichia coli* O157:H7 to lettuce leaf surface and bacterial viability in response to chlorine treatment. Journal of Food Protection 62 (1), 3–9.

Solomon, E.B., Pang, H.J., Matthews, K.R., 2003. Persistence of *Escherichia coli* O157:H7 on lettuce plants following spray irrigation with contaminated water. Journal of Food Protection 66 (12), 2198–2202.

Wachtel, M.R., Charkowski, A.O., 2002. Cross-contamination of lettuce with *Escherichia coli* O157:H7. Journal of Food Protection 65 (3), 465–470.

Wachtel, M.R., Whitehand, L.C., Mandrell, R.E., 2001. Association of *Escherichia coli* O157:H7 with pre-harvest leaf lettuce upon exposure to contaminated irrigation water. Journal of Food Protection 65 (1), 18–25.

Wachtel, M.R., Whitehand, L.C., Mandrell, R.E., 2002. Prevalence of *Escherichia coli* associated with a cabbage crop inadvertently irrigated with partially treated sewage wastewater. Journal of Food Protection 65 (3), 71–475.

Wachtel, M.R., McEvoy, J.L., Luo, Y., Williams-Campbell, A.M., Solomon, M.B., 2003. Cross-contamination of lettuce (*Lactuca sativa* L.) with *Escherichia coli* O157:H7 via contaminated ground beef. Journal of Food Protection 66 (7), 1176–1183.

Wells, J.M., Butterfield, J.E., 1997. Salmonella contamination associated with bacterial soft rot of fresh fruits and vegetables in the marketplace. Plant Disease 81 (8), 867–872.