Journal of Food Protection, Vol. 77, No. 5, 2014, Pages 752–757
doi:10.4315/0362-028X.JFP-13-370

Salmonella Internalization in Mung Bean Sprouts and Pre- and Postharvest Intervention Methods in a Hydroponic System

CHONGTAO GE,[1]† SUSAN RYMUT,[1]† CHEONGHOON LEE,[2] AND JIYOUNG LEE[1,2]*

[1]*Department of Food Science and Technology and* [2]*College of Public Health, Division of Environmental Health Sciences, The Ohio State University, Columbus, Ohio 43210, USA*

MS 13-370: Received 6 September 2013/Accepted 11 December 2013

ABSTRACT

Mung bean sprouts, typically consumed raw or minimally cooked, are often contaminated with pathogens. Internalized pathogens pose a high risk because conventional sanitization methods are ineffective for their inactivation. The studies were performed (i) to understand the potential of internalization of *Salmonella* in mung bean sprouts under conditions where the irrigation water was contaminated and (ii) to determine if pre- and postharvest intervention methods are effective in inactivating the internalized pathogen. Mung bean sprouts were grown hydroponically and exposed to green fluorescence protein–tagged *Salmonella* Typhimurium through maturity. One experimental set received contaminated water daily, while other sets received contaminated water on a single day at different times. For preharvest intervention, irrigation water was exposed to UV, and for postharvest intervention–contaminated sprouts were subjected to a chlorine wash and UV light. Harvested samples were disinfected with ethanol and $AgNO_3$ to differentiate surface-associate pathogens from the internalized ones. The internalized *Salmonella* Typhimurium in each set was quantified using the plate count method. Internalized *Salmonella* Typhimurium was detected at levels of 2.0 to 5.1 log CFU/g under all conditions. Continuous exposure to contaminated water during the entire period generated significantly higher levels of *Salmonella* Typhimurium internalization than sets receiving contaminated water for only a single day ($P < 0.05$). Preintervention methods lowered the level of internalized *Salmonella* by 1.84 log CFU/g ($P < 0.05$), whereas postintervention methods were ineffective in eliminating internalized pathogens. Preintervention did not completely inactivate bacteria in sprouts and demonstrated that the remaining *Salmonella* Typhimurium in water became more resistant to UV. Because postharvest intervention methods are ineffective, proper procedures for maintaining clean irrigation water must be followed throughout production in a hydroponic system.

Sprouts are becoming increasingly popular due to their high vitamin and mineral content, leading to associated health benefits *(25, 30)*. It is estimated by industry experts that 15 million pounds of sprouts are grown annually in the United States. Mung bean has been widely grown, especially in Asian countries since ancient times and is still widely grown in Asia, Africa, South America, the United States, and Australia. Mung bean is best known for the production of bean sprouts. It is very convenient to produce mung bean sprouts at home or industry scale: briefly, soak the mung bean seeds in water overnight at room temperature until the seeds germinate; transfer the germinated seeds to a bigger container; and sprinkle water during the sprouting process. The sprouts are ready to consume after 4 to 5 days.

However, the sprouts are vulnerable to microbial contamination because they are grown in a nutrient-rich and moist environment *(13, 40)*. A previous study has shown that aerobic microorganisms were increased by 3 log CFU/g during the sprouting stage, and several foodborne pathogens were isolated at the final stage of production *(26)*. Because sprouts are typically consumed raw, they pose a high health risk to consumers as a vehicle for foodborne disease *(43)*. A number of sprout-linked foodborne outbreaks have been reported since the 1990s, and *Salmonella* has been one of the most commonly associated pathogens, with the primary source of the pathogen being either contaminated seeds or irrigation water *(31, 33, 34, 38)*.

Pathogen contamination of sprouts can occur during the production process at a manufacturing site. For example, the *Salmonella* Enteritidis outbreak associated with mung bean sprouts in Canada was likely because the manufacturing site was contaminated by the bacteria from the adjacent poultry processing plant *(42)*. In addition, water used for production might also be responsible for contamination of sprouts. Furthermore, when contaminated water comes into contact with growing produce, it has the capacity to internalize pathogens in the plant. Bacteria can enter a plant via the root system, stomata, or cracks in the leafy green *(28, 35)*. If the germination of seeds and further production of green sprouts are conducted in a hydroponic growing system, where the water is potentially polluted, an understanding of the ability of *Salmonella* to internalize in sprouts must be determined and methods must be developed to prevent such events.

Seed treatment is considered as a critical barrier against microbial contamination of edible plants. The U.S. Food

* Author for correspondence. Tel: 614-292-5546; Fax: 614-292-4053; E-mail: lee.3598@osu.edu.
† Both authors contributed equally.

and Drug Administration recommends soaking the seeds by adding 20,000 ppm of calcium hypochlorite for 15 min before proceeding to the sprouting phase *(44)*. Much research has focused on creating novel methods of killing pathogens in sprout seeds, but fewer inquiries have focused on contaminated irrigation water *(2, 24, 29)*. In addition, once microbial internalization occurs during the sprouting stage, the use of external washes with chemical disinfectants is ineffective in inactivating the microorganisms inside the plants *(5–7, 39)*. Among the studies that have attempted to inactivate internalized microorganisms, gamma irradiation has proved to be highly efficient *(17, 36)*. However, although gamma-radiated food has been proven to be safe for human consumption, the public perception to the safety of irradiated food has not been improved *(32)*. Therefore, the U.S. Food and Drug Administration requires that any gamma-radiated food should be labeled with the ''radura'' logo and with a disclosure statement included that reads ''Treated with radiation'' or ''Treated by irradiation'' *(45)*. Additionally, the cost and safety issues of ionizing irradiation may lower the perceived value of its application in food preservation *(11)*. As a method for nonionizing irradiation, UV-C (200 to 280 nm, which has strong germicidal ability) has been used for microbial disinfection of water, work surfaces, and food products for decades *(18)*. Furthermore, UV-C light has been found to be successful in reducing internalized pathogens, such as *Salmonella* and *Escherichia coli* O157:H7, when bacteria are near the surface *(15, 20, 48)*.

The current study first examined the levels of internalized *Salmonella* in sprouts grown hydroponically under conditions in which the time and length of exposure of sprouts to bacteria were varied. Secondly, preharvest UV treatment of irrigation water and postharvest chlorine washes of sprouts and/or UV treatment were used as disinfection methods to determine whether these interventions were useful in preventing or eliminating pathogen internalization. This study offers valuable information to sprout growers and food industry providers about methods to inhibit bacterial internalization and thus ensure the health and safety of consumers.

MATERIALS AND METHODS

Hydroponic growth of mung bean sprout. Seeds of mung bean (*Vigna radiata*) were purchased from a local grocery store and were germinated in sterilized tap water in petri dishes at 20 to 22°C in the dark, with a beans-to-water ratio of 1:5 (wt/vol; each petri dish contained ~6 g of seeds soaking in 30 ml of water). The water drained after 12 h. About 20 g of germinated sprouts were added in a small plastic bucket (250-ml capacity, inner bucket) with holes at the bottom and then suspended inside a larger bucket (3-liter capacity, outer bucket) through tubing. For contaminating the irrigation water, *Salmonella* Typhimurium (ATCC 19585; purchased from American Type Culture Collection) was labeled with green fluorescence protein, as described in our previous study *(16)*. The green fluorescence protein–tagged *Salmonella* Typhimurium was cultured overnight and then added to 2 liters of irrigation water to reach a final concentration of 10^9 CFU/ml. The contaminated water was reserved in the outer bucket and sprayed over the sprouts via tubing at a rate of 4.2 liters/min for 1 min with a portable air pump. Water was allowed to completely drain from the sprouts and into the outer bucket. This cycle of watering and draining occurred every 8 h for 5 to 6 days, and the sprouts were grown in a 20 to 22°C dark room at 30 to 40% relative humidity.

Determination of *Salmonella* in mung bean sprout. Mature mung bean sprouts were harvested, and hulls were removed using sterile forceps. Prior to determination of the internalized pathogen, the surfaces were disinfected with 80% ethanol for 10 s, 1% $AgNO_3$ for 5 min, and rinsed in deionized water *(14)*. The surface-decontaminated sprouts were homogenized in 0.1% peptone water (wt/vol) for 2 min in a sterile Whirl-Pak bag (Nasco, Fort Atkinson, WI). The homogenized solution was directly plated onto Luria-Bertani agar supplemented with 100 μg/ml (final concentration) ampicillin and incubated at 37°C for ~18 h. The plates were examined for internalized green fluorescence protein *Salmonella* Typhimurium colonies on the agar plates under UV light (365 nm) prior to enumeration. Each set of the sprout samples (10 g of mung bean sprouts) was tested in duplicate and all experiments were repeated in triplicate.

Time dependency of internalization. *Salmonella* Typhimurium was added to the irrigation water at a final concentration of 9 log CFU/ml on varying days to determine the temporal relationship between contact with the pathogen and the level of internalization upon harvest. *Salmonella* Typhimurium was added to the irrigation water on one of the days after germination. The UV-C–treated irrigation water was circulated on day 1, 3, 4, 5, or 6 or every day (days 1 to 6) throughout the growth period. Sprouts were grown for a total of 6 days and watered every 8 h. Samples were collected once every 24-h cycle for the detection of internalized pathogen.

Preharvest intervention. A UV-C irradiation chamber was constructed for food and water disinfection, as described in our previous study *(15)*. Briefly, two UV-C lamps (254 nm, 15 W; Philips TUV15 W G15T8 Long L, Philips, Eindhoven, The Netherlands) were located on the top inside a plywood chamber, and the UV intensity was measured by a UV-C meter (UV512C, Digital UV-C Meter; General Tools, New York, NY). The UV-C fluency (millijoules per square centimeter) was calculated as intensity × time. Irrigation water was contaminated with 9 log CFU/ml *Salmonella* Typhimurium. Before exposing the sprouts to the irrigation water and every 24 h throughout growth, water (~20 cm in depth) was subjected to UV-C light (UV fluency = 950 mJ/cm^2, 1.583 mW/cm^2 for 10 min). The UV-C–treated irrigation water was circulated in the sprout growing system, and irrigation water samples were also collected daily before and after UV irradiation to determine the viable remaining *Salmonella* Typhimurium. Sprouts were harvested after 6 days of growth (on day 7).

Postharvest intervention. Mung bean sprouts were grown with the contaminated water (9 log CFU/ml *Salmonella* Typhimurium) for 7 days as described above. The water was recycled during the entire length of growth. When harvested, the surfaces were disinfected, and the sprouts were given one of the postharvest interventions: (i) UV-C irradiation, (ii) a chlorine wash (500, 1,000, or 2,000 mg/liter/min; Table 1), or (iii) a combined chlorine wash (2,000 mg/liter/min) followed by UV irradiation (778 mJ/cm^2) in an effort to remove the internalized pathogen. The sprouts was spread on a tray as a monolayer, and 100 g of sprouts was prepared to be treated at a time. The UV-C radiation fluency varied by adjusting the distance between the UV-C lamp and the sprouts.

TABLE 1. *Postharvest treatments of mung bean sprouts using UV-C irradiation and chlorine wash*

Treatment	Intensity (mW/cm^2)	Treatment time (min)	Fluency (mJ/cm^2)
UV-C	0.130	10	78
	0.260	10	156
	1.296	10	778
	Concn (mg/liter)	Treatment time (min)	C × *t* value (mg/liter/min)
Chlorine	50	10	500
	100	10	1,000
	200	10	2,000

The chlorine wash was prepared by adding bleach (71.42 mg/ml; Clorox, Oakland, CA) to deionized water, and about 100 g of sprouts was immersed in chlorinated water (1 liter) in each treatment.

Statistical analysis. The statistical analysis was carried out with SPSS 17.0 statistical software (SPSS Inc., Chicago, IL). Arithmetic means and standard deviations of all the data of the internalized *Salmonella* Typhimurium and the *Salmonella* Typhimurium concentration in the irrigation water were calculated. Analysis of variance and Dunnett's test were applied to evaluate differences, and the results were considered significant at $P < 0.05$.

RESULTS

Time dependency of *Salmonella* internalization. *Salmonella* Typhimurium internalization was present in every group of sprout whether by a continuous regimen or 1-day contamination (Fig. 1). The level of internalization ranged from 3 to 5 CFU/g in sprouts exposed throughout the experiment and 2 to 5 CFU/g in sprouts exposed to bacteria for only 1 day during the growth period. The ability of *Salmonella* Typhimurium to internalize in mung bean sprouts was not dependent on the day of exposure during which bacteria were in contact with the sprouts (Fig. 1). When sprouts were in contact with contaminated water daily, the level of internalization in the harvested sprouts (on day 7) was significantly greater than when sprouts were exposed for only 1 day during growth ($P < 0.05$). Sprouts in contact with *Salmonella* Typhimurium for only 1 day during growth did not show a significant decrease ($P > 0.05$) in the level of bacteria from the initial exposure day to the final harvest day (on day 7). Similarly, there was no significant difference in levels of internalization between sprouts at harvest that were contaminated on day 1, 3, 4, 5, or 6.

Preharvest intervention. *Salmonella* Typhimurium in the irrigation water was significantly decreased after exposure to UV light on each day (Table 2; $P < 0.05$). However, in the next 24-h period after UV, the level of *Salmonella* Typhimurium was found to be 4 to 5 log CFU/ml, and the level of the remaining *Salmonella* Typhimuriun in the water slightly increased after UV intervention (1.95 to 2.98 log CFU/ml from day 2 to day 5, $P > 0.05$). In water with no UV irradiation, the concentration of *Salmonella* Typhimurium in irrigation water decreased ~1 log daily.

Exposure of contaminated water to 950 mJ/cm^2 UV once before watering resulted in a significant decrease ($P < 0.05$) in the amount of internalized *Salmonella* Typhimurium in mung bean sprouts (Table 3). Daily preharvest UV irradiation resulted in a ~2-log reduction in internalized pathogens compared with sprouts with no intervention. However, the level of internalized *Salmonella* Typhimurium remained high (3.72 log CFU/g).

Postharvest intervention. From the results of the plate counts, it was found that *Salmonella* Typhimurium was internalized in the mung bean sprout regardless of treatment with chlorine, UV-C light, or a combination of chlorine and UV-C (Table 4). There was no significant difference ($P > 0.05$) in the level of internalization between any of the treatments. Furthermore, there was no decrease in the level of *Salmonella* internalization with the doses of UV-C that were used.

DISCUSSION

Pathogen internalization has been found in a variety of fruits and vegetables in numerous studies *(4, 10, 19, 22)*. Specifically, Itoh et al. *(23)* demonstrated that *E. coli* O157 was able to internalize throughout the whole edible portion of radish sprouts when the roots were immersed in contaminated water. Both *Salmonella* Montevideo and *E. coli* were capable of being internalized into growing sprout when seeds were inoculated and grown on soft agar beds *(46)*. Andrews et al. *(1)* proposed that it was exudates

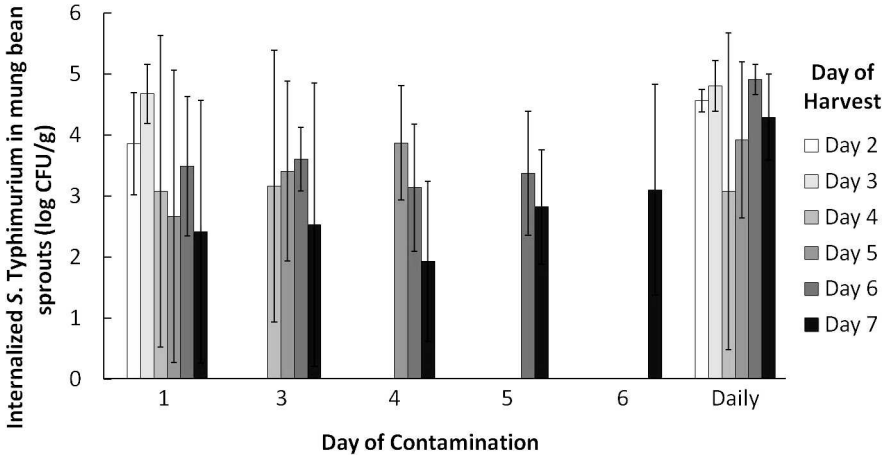

FIGURE 1. *Level of internalization of* Salmonella *Typhimurium in mung bean sprouts when in contact with the pathogen on days 1, 3, 4, 5, 6, or daily of growth and harvested 24 h after the initial contamination.*

TABLE 2. *The levels of* Salmonella *Typhimurium in irrigation water before and after exposure to UV-C light and water without exposure to UV-C light over a 24-h period used in sprout growth*

	Salmonella Typhimurium (log CFU/ml) on irrigation day:				
	2	3	4	5	6
Without UV treatment	9.24 ± 0.21	8.04 ± 0.12	6.87 ± 0.15	4.81 ± 0.14	4.75 ± 0.28
With UV treatment					
Before	9.24 ± 0.21	5.06 ± 0.58	5.09 ± 0.18	4.09 ± 0.33	3.88 ± 0.31
After	1.95 ± 1.27	1.40 ± 0.98	2.36 ± 0.36	2.62 ± 0.31	2.98 ± 0.62

released from the germinating seeds that attracted and supported bacterial growth around the vicinity of the plants during the early sprouting stage. The internalization of bacteria into the sprouts can occur through cracks in the epidermis and fissures of plants and disseminated into the entire developed hypocotyls *(3, 46)*. In the current study, it was examined if the level of internalization was dependent upon the day that the pathogen first came in contact with the sprouts and if there is any difference between daily exposures to the pathogen versus a one-time exposure during growth. As shown in Figure 1, internalized *Salmonella* Typhimurium was detectable the day after exposure to contaminated water. Moreover, the internalized *Salmonella* Typhimurium did not disappear (e.g., level of internalized bacteria was more than 2 log CFU/g when the sprouts were collected on day 7 following exposure to *Salmonella* Typhimurium on day 1). Based upon these results, it is posited that microbial internalization may occur whenever sprouts are exposed to contaminated water, and these scenarios will pose a health risk when sprouts are consumed.

The most commonly used method for intervention, washing with chlorine, has been shown ineffective for inactivation of internalized pathogens in fresh produce *(8, 39)*. Studies have demonstrated that using ionizing irradiation, such as gamma ray or electric beam, may significantly decrease internalized bacteria in plants *(9, 17, 36)*. However, issues, such as cost and maintenance of the irradiation processing equipment and consumer perception of the safety of irradiated food, limit the use of ionizing irradiation in food industry *(11, 45, 32, 37)*. Treating fresh produce with UV-C, as a nonionizing irradiation method, was found to significantly decrease internalized pathogens in lettuce and other leafy greens, suggesting UV-C as a possible method to decrease the microbial load internalized in bean sprouts *(15, 20)*. UV has been widely used for decontaminating air, water, and food for several decades *(12, 18)*. Combining UV-C light (37.8 mJ/cm^2) with hydrogen peroxide (1.5%, wt/liter, 60 s) was able to remove 2.84 log CFU/g internalized *Salmonella* Montevideo P2 in lettuce *(20)*. Our previous study showed that 1.96 and 1 log reductions of internalized *Salmonella* Typhimurium were achieved when using 150 mJ/cm^2 UV-C to treat lettuce and green onion, respectively *(15)*. In the current study, different fluencies of UV-C and different concentrations of chlorine (as a comparison) were ineffective in inactivating internalized *Salmonella* Typhimurium; no significant reduction was observed even when UV-C fluency reached 778 mJ/cm^2 (Table 4; $P > 0.05$). Neither chorine washing nor a combination of chlorine washes followed by high fluency UV-C treatment could achieve a significant reduction of internalized *Salmonella* Typhimurium in mung bean sprouts ($P > 0.05$).

Preharvest intervention with UV light significantly decreased the level of pathogen in the irrigation water and the level of bacteria internalized in sprouts ($P < 0.05$) (Table 2). The 24-h period treatment of water with 950 mJ/cm^2 UV-C was able to reduce the internalized *Salmonella* Typhimurium by 1.84 log CFU/g (Table 3). On day 1, a 7.3-log reduction of *Salmonella* was achieved in the irrigation water, but the level of inactivation was less after day 1 (3.66 log on day 2 to 0.89 log on day 5), and the remaining bacteria in the irrigation water actually increased from day 2 to day 5). These results indicated that *Salmonella* Typhimurium (in the irrigation water) not only recovered, but became more resistant to UV treatment. Exposure of bacteria, including *Salmonella,* to UV light may cause mutations and increase the efficiency of UV repair, making the bacteria more resistant to subsequent UV

TABLE 3. *The levels of internalized* Salmonella *Typhimurium in the sprouts that were irrigated with contaminated water without treatment or with UV-C preintervention*

	Internalized *Salmonella* Typhimurium (log CFU/g)
No treatment	5.56 ± 0.31
Preintervention	3.72 ± 0.60

TABLE 4. *The levels of internalized* Salmonella *Typhimurium in mung bean sprouts after exposure to chlorine wash, UV light, and combination of chlorine and UV*

	Internalized S*almonella* Typhimurium (log CFU/g)
No treatment	4.87 ± 0.78
UV-C treatment (mJ/cm^2)	
78	5.13 ± 0.50
156	5.46 ± 0.35
778	5.26 ± 0.44
Chlorine treatment (mg/liter/min)	
500	4.83 ± 0.86
1,000	5.02 ± 0.58
2,000	4.19 ± 0.62
Combined treatment (778 mJ/cm^2 UV + 2,000 mg/liter/min chlorine)	4.71 ± 0.81

exposure *(18, 26, 27, 41)*. In addition, dissolved organic nutrients released from sprouting could be a shield or absorb UV light that lower the efficacy of UV disinfection *(21, 47)*.

Overall, *Salmonella* Typhimurium internalizes in mung bean sprouts, which is not dependent upon the stage of growth of the sprout. Sprouts continuously contaminated with *Salmonella* Typhimurium showed a significantly higher level of internalization when compared with sprouts exposed only one time during growth. Postharvest intervention methods were not effective in reducing the level of internalized pathogen, whereas preharvest intervention using UV-C light to decontaminate irrigation water significantly decreased the level of internalized pathogen, although internalized *Salmonella* Typhimurium was still detectable. *Salmonella* Typhimurium was able to recover in the irrigation water over a 24-h period and became more resistant to UV-C light. Based on our finding, it is recommended that producers monitor their water source prior to use on growing sprouts to prevent foodborne disease outbreaks, because internalization is inevitable in the presence of pathogens such as *Salmonella*. Once the sprouts contain pathogens, they cannot be fully inactivated even when using postharvest UV-C and chlorine intervention methods.

ACKNOWLEDGMENT

This study was partially supported by funds provided by the Public Health Preparedness for Infectious Diseases program (phpid.osu.edu) of The Ohio State University.

REFERENCES

1. Andrews, W. H., P. B. Mislivec, C. R. Wilson, V. R. Bruce, P. L. Poelma, R. Gibson, M. W. Trucksess, and K. Young. 1982. Microbial hazards associated with bean sprouting. *J. Assoc. Off. Anal. Chem.* 652:241–248.
2. Bari, L., K. Enomoto, D. Nei, and S. Kawamoto. 2010. Scale-up seed decontamination process to inactivate *Escherichia coli* O157:H7 and *Salmonella* Enteritidis on mung bean seeds. *Foodborne Pathog. Dis.* 7:51–56.
3. Barraquio, W. L., L. Revilla, and J. K. Ladha. 1997. Isolation of endophytic diazotrophic bacteria from wetland rice. *Plant Soil* 194: 15–24.
4. Bernstein, N., S. Sela, R. Pinto, and M. Ioffe. 2007. Evidence for internalization of *Escherichia coli* into the aerial parts of maize via the root system. *J. Food Prot.* 70:471–475.
5. Beuchat, L. R. 1998. Surface decontamination of fruits and vegetables eaten raw: a review. Food Safety Unit, WHO/FSF/FOS/ 98.2. World Health Organization, Geneva.
6. Beuchat, L. R., L. J. Harris, T. E. Ward, and T. M. Kajs. 2001. Development of a proposed standard method for assessing the efficacy of fresh produce sanitizers. *J. Food Prot.* 64:1103–1109.
7. Beuchat, L. R., B. V. Nail, B. B. Adler, and M. R. S. Clavero. 1998. Efficacy of spray application of chlorinated water in killing pathogenic bacteria on raw apples, tomatoes, and lettuce. *J. Food Prot.* 61:1305–1311.
8. Chaidez, C., M. Moreno, W. Rubio, M, Angulo, and B. Valdez. 2003. Comparison of the disinfection efficacy of chlorine-based products for inactivation of viral indicators and pathogenic bacteria in produce wash water. *Int. J. Environ. Health Res.* 13:295–302.
9. Chimbombi, E., R. G. Moreira, J. Kim, and E. M. Castell-Perez. 2011. Prediction of targe*ted Salmonella enterica* serovar Typhimurium inactivation in fresh cut cantaloupe (*Cucumis melo* L.) using electron beam irradiation. *J. Food Eng.* 103:409–416.
10. Deering, A. J., L. J. Mauer, and R. E. Prutt. 2012. Internalization of *E. coli* O157:H7 and *Salmonella* spp. in plants: a review. *Food Res. Int.* 45:567–575.
11. Durante, R. W. 2002. Food processors requirements met by radiation processing. *Radiat. Phys. Chem.* 63:289–294.
12. Environmental Protection Agency. 1996. Ultraviolet light disinfection technology in drinking water application: an overview. Available at: http://nepis.epa.gov. Accessed 1 June 2012.
13. Fahey, J. W., P. J. Ourisson, and F. H. Degnan. 2006. Pathogen detection, testing, and control in fresh broccoli sprouts. *Nutr. J.* 5:13.
14. Franz, E., A. A. Visser, A. D. Van Diepeningen, M. M. Klerks, A. J. Termorshuizen, and A. H. C. van Bruggen. 2007. Quantification of contamination of lettuce by GFP-expressing Escherichia coli O157:H7 and Salmonella enterica serovar Typhimurium. *Food Microbiol.* 24:106–112.
15. Ge, C., A. Bohrerova, and J. Lee. 2013. Inactivation of internalized *Salmonella* Typhimurium in lettuce and green onion using ultraviolet C irradiation and chemical sanitizers. *J. Appl. Microbiol.* 114:1415–1424.
16. Ge, C., C. Lee, and J. Lee. 2012. The impact of extreme weather events on *Salmonella* internalization in lettuce and green onion. *Food Res. Int.* 45:1118–1122.
17. Gomes, C., P. Da Silva, R. G. Moreira, E. Castell-Perez, E. A. Ellis, and M. Pedlenton. 2009. Understanding *E. coli* internalization in lettuce leaves for optimization of irradiation treatment. *Int. J. Food Microbiol.* 135:238–247.
18. Guerrero-Beltrán, J. A., and G. V. Barbosa-Cánovas. 2004. Advantages and limitations on processing foods by UV light. *Food Sci. Technol. Int.* 10:137–147.
19. Guo, X., M. W. van Iersel, J. Chen, R. E. Brackett, and L .R. Beuchat. 2002. Evidence of association of *Salmonellae* with tomato plants grown hydroponically in inoculated nutrient solution. *Appl. Environ. Microbiol.* 68:3639–3643.
20. Hadjok, C., G. S. Mittal, and K. Warriner. 2008. Inactivation of human pathogens and spoilage bacteria on the surface and internalized within fresh produce by using a combination of ultraviolet light and hydrogen peroxide. *J. Appl. Microbiol.* 104: 1014–1024.
21. Howard M. B., and S. W. Hutcheson. 2003. Growth dynamics of *Salmonella enterica* stains on alfalfa sprouts and in waste seed irrigation water. *Appl. Environ. Microbiol.* 69:548–553.
22. Ibarra-Sánchez, L. S., S. Alvarado-Casillas, M. O. Rodríguez-García, N. E. Martínez-Gonzáles, and A. Castillo. 2004. Internalization of bacterial pathogens in tomatoes and their control by selected chemicals. *J. Food Prot.* 67:1353–1358.
23. Itoh, Y., Y. Sugita-Konishi, F. Kasuga, M. Iwaki, Y. Hara-Kudo, N. Saito, Y. Noguchi, H. Konuma, and S. Kumagai. 1998. Enterohemorrhagic *Escherichia coli* O157:H7 present in radish sprouts. *Appl. Environ. Microbiol.* 64:1532–1535.
24. Jin, H.-H., and S.-Y. Lee, 2007. Combined effect of aqueous chlorine dioxide and modified atmosphere packaging on inhibiting *Salmonella* Typhimurium and *Listeria monocytogenes* in mungbean sprouts. *J Food Sci.* 72:M441–M445.
25. Jung, W. Y., Y. M. Choi, and M. S. Rhee. 2009. Potential use of supercritical carbon dioxide to decontaminate *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Salmonella* Typhimurium in alfalfa sprouted seeds. *Int. J. Food Microbiol.* 136:66–70.
26. Kim, S. A., O. M. Kim, and M. S. Rhee. 2013. Changes in microbial contamination levels and prevalence of foodborne pathogens in alfalfa (*Medicago sativa*) and rapeseed (*Brassica napus*) during sprout production in manufacturing plants. *Lett. Appl. Microbiol.* 56: 30–36.
27. Kramer, G. F., J. C. Baker, and B. N. Ames. 1988. Near-UV stress in *Salmonella typhimurium*: 4-Thiouridine in tRNA ppGpp, and ApppGpp as components of an adaptive response. *J. Bacteriol.* 170: 2344–2351.
28. Kroupitski, Y., D. Goldberg, E. Belausov, R. Pinto, D. Swartzberg, D. Granot, and S. Sela. 2009. Internalization of *Salmonella enterica* in leaves is induced by light and involves chemotaxis and penetration through open stomata. *Appl. Environ. Microbiol.* 75:2076–6086.

29. Kumar, M., R. Hora, M. Kostrzynska, W. M. Waites, and K. Warriner. 2006. Inactivation of *Escherichia coli* 0157:H7 and *Salmonella* on mung beans, alfalfa, and other seed types destined for sprout production by using an oxychloro-based sanitizer. *J. Food Prot.* 69:1571–1578.
30. Kylen, A. M, and R. M. McCready. 1975. Nutrients in seeds and sprouts of alfalfa, lentils, mung beans, and soybeans. *J. Food Sci.* 40: 1008–1009.
31. Liu, B., and D. W. Schaffner. 2006. Quantitative analysis of the growth of *Salmonella* Stanley during alfalfa sprouting and evaluation of *Enterobacter aerogenes* as its surrogate. *J. Food Prot.* 70:316–22.
32. Mahapatra, A. K., K. Muthukumarappan, and J. L. Julson. 2005. Applications of ozone, bacteriocins and irradiation in food processing: a review. *Crit. Rev. Food Sci. Nutr.* 45:447–461.
33. Mohle-Boetani, J. C., J. A. Farrar, P. Bradley, J. D. Barak, M. Miller, R. Mandrell, P. Mead, W. E. Keene, K. Cummings, S. Abbott, and S. B. Werner. 2009. *Salmonella* infections associated with mung bean sprouts: epidemiological and environmental investigations. *Epidemiol. Infect.* 137:357–366.
34. Mohle-Boetani, J. C., J. A. Farrar, S. B. Warner, D. Minassian, R. Bryant, S. Abbott, L. Slutskert, and D. J. Vugia. 2001. *Escherichia coli* O157 and *Salmonella* infections associated with sprouts in California, 1996–1998. *Ann. Intern. Med.* 135:239–247.
35. Natvig, E. E., S. C. Ingham, B. H. Ingham, L. R. Cooperband, and T. R. Roper. 2002. *Salmonella enterica* Serovar Typhimurium and *Escherichia coli* contamination of root and leaf vegetables grown in soils with incorporated bovine manure. *Appl. Environ. Microbiol.* 68: 2737–2744.
36. Niemira, B. A. 2008. Irradiation compared with chlorination for elimination of *Escherichia coli* O157:H7 internalized in lettuce leaves: influence of lettuce variety. *J. Food Sci.* 73:M208–M213.
37. O'Bryan, C. A., P. G. Crandall, S. C. Ricke, and D. G. Olson. 2008. Impact of irradiation on the safety and quality of poultry and meat products: a review. *Crit. Rev. Food Sci. Nutr.* 48:442–457.
38. Powell, D. 2005. Sprouts associated outbreaks in North America. Commentary from the food safety network. Available at: http://www.foodsafetynetwork.ca. Accessed on 19 February, 2013.
39. Sapers, G. M. 2001. Efficacy of washing and sanitizing methods. *Food Technol. Biotechnol.* 39:305–311.
40. Schrader, W. L. 2002. Sprout production in California. Agriculture and Natural Resources, University of California, Davis. Publication 8060. Available at: http://anrcatalog.ucdavis.edu/Items.aspx?search=sprout. Accessed 26 November 2013.
41. Sommer, R., M. Lhotsky, T. Haider, and A. Cabaj. 2000. UV inactivation liquid-holding recovery and photoreactivation *of Escherichia coli* O157 and other *Escherichia coli* pathogenic strains in water. *J. Food Prot.* 63:1015–1020.
42. Sproutnest. 2005. Outbreak of *Salmonella* Enteritidis Phage Type 13 associated with mung bean sprouts in Ontario, 2005. Available at: http://www.sproutnet.com/pdfs/Toronto-Mung-2005.pdf. Accessed 2 November 2013.
43. Taormina, P. J., L. R. Beuchat, and L. Slutsker. 1999. Infections associated with eating seed sprouts: an international concern. *Emerg. Infect. Dis.* 5:626–634.
44. U.S. Food and Drug Administration. 1999. Federal Register Advance Notice of Proposed Rulemaking—62 FR 7834 February 17, 1999—Irradiation in the production, processing, and handling of food. U.S. Food and Drug Administration, Washington, DC.
45. U.S. Food and Drug Administration. 1999. Guidance for industry: sampling and microbial testing of spent irrigation water during sprout production. *Fed. Regist.* 64:57893–57902.
46. Warriner, K., S. Spaniolas, M. Dickinson, C. Wright, and W. M. Waites. 2003. Internalization of bioluminescent *Escherichia coli* and *Salmonella Montevi*deo in growing bean sprouts. *J. Appl. Microbiol.* 95:719–727.
47. World Health Organization. 2002. Water sanitation health. Managing water in the home: accelerated health gains from improved water supply: heat and UV radiation. Available at: http://www.who.int/water_sanitation_health/dwq/wsh0207/en/index4.html. Accessed 2 November 2013.
48. Yaun, B. R., S. S. Sumner, J. D. Eifert, and J. E. Marcy. 2004. Inhibition of pathogens on fresh produce by ultraviolet energy. *Int. J. Food Microbiol.* 90:1–8.