Rajalingam *et al. Appl Biol Chem* (2021) 64:29
https://doi.org/10.1186/s13765-021-00597-3

KSABC
The Korean Society for Applied Biological Chemistry

NOTE

Open Access

Prevalence and molecular characterization of *Escherichia coli* isolates during radish sprout production in the Republic of Korea

Nagendran Rajalingam[1], Jae-Hyun Yoon[1], Bohyun Yoon[2], Nguyen Bao Hung[1,5], Won-Il Kim[1], Hyunju Kim[3], Byeong Yong Park[4] and Se-Ri Kim[1,6*]

Abstract

This study was conducted to investigate the prevalence of coliforms and *Escherichia coli* across radish sprout production facilities in the Republic of Korea and to characterize isolated *E. coli*. A total of 324 samples were collected from three different radish sprout production units in spring and summer. Overall, the levels of coliforms were significantly higher (> 3.75 log CFU/g) in radish sprouts during summer season than in spring. *E. coli* was detected in the pre-harvest (50%) and post-harvest (39%) sprouts and water samples (33%) from farms B and C. Of the 48 *E. coli* isolates, five *E. coli* isolates were positive for the *eaeA* gene, indicative of EPEC O110, two isolates were *eaeA*-positive (EPEC ONT) and one isolate was *ipaH*-positive (ETEC O71). These three *E. coli* strains were identified as novel sequence types by the multilocus sequence-typing assay based on the combination of allelic profiles. In addition, the EPEC O110 isolates obtained from the stream water stored in container of farm C were toxic to *Caenorhabditis elegans*. It is necessary to implement safety measures during the production of radish sprouts to prevent *E. coli* outbreaks.

Keywords: *Escherichia coli*, Radish sprout, Multilocus sequence typing (MLST), Toxicity

Introduction

Due to consumer demands for healthy and natural food products, seed sprouts with antioxidant and anti-tumor properties have gained worldwide popularity [1, 2]. This growing demand has been accompanied by an increase in bacterial outbreaks associated with sprouted seed products [3].

In the last four decades, several outbreaks linked with seed sprouts have been reported in several countries [4]. The world's largest foodborne outbreak was caused by *Escherichia coli* O157:H7, which involved more than 7,000 patients, was associated with white radish sprout consumption in Japan [5]. A massive outbreak of EHEC O104:H4 linked with the fenugreek sprouts caused illness in 6,829 patients and 53 deaths in Germany during the summer of 2011 [6]. In the same year, 29 people were infected with EHEC O26 after consuming raw clover sprouts served in the Jimmy John's restaurant, USA [7]. Moreover, a multistate EHEC O103 outbreak involving 51 people linked to clover sprouts was reported in the USA [8].

Microbial contamination in sprouts can occur from numerous sources, including contaminated seeds, soil, fertilizer, tools, and the water used during the pre- and post-harvest processing stages [9]. Preharvest sprout contamination may occur from substandard water, soil, and the close proximity of cattle rearing facilities [10], while postharvest contamination may occur during packing, transport, and storage [11, 12]. Coliforms are group of closely related harmless bacteria that are widespread throughout the environment including soil and water. The level of coliform bacteria indicates the microbial

*Correspondence: seri81@korea.kr
[6] Agro-Food Safety & Crop Protection Department, National Institution of Agricultural Science, Rural Development Administration, Wanju 55365, South Korea
Full list of author information is available at the end of the article

quality of the food product and generally reflects the hygienic practices followed during food production or handling [13].

Laboratory-based monitoring of sprout producing units and the frequent safety evaluation of the farm tools and materials used during the production process can help to identify the source of contamination and prevent disease outbreaks. Thus, we collected samples from all the materials used in sprout production, including seeds, sprouts, water, farming, and packing tools from various sprout production companies and analyzed them for the presence of pathogenic *E. coli*. The isolated strains were discriminated into subgroups by serotyping and further characterized at the molecular level using multiplex polymerase chain reaction (PCR) and multilocus sequence typing (MLST) and genetic relatedness between the isolates. In addition, the toxicity of the isolates was determined using *Caenorhabditis elegans*. The results of this study provide information about the prevalence and characteristics of *E. coli* isolates recovered from radish sprout production environments.

Materials and methods

Sampling

Three hundred twenty-four samples, including radish seeds (n = 18), preharvest sprouts (n = 54), postharvest sprouts (n = 18), ground water pumped from well (n = 18), irrigation water i.e. water passed from well through pipes to sprouts directly (n = 18), stream water (n = 6). Agricultural materials nutrient solutions A (n = 18), B (n = 18) and A + B (n = 18), cultivation equipments including nutrients storage tank (n = 18), fabric (n = 18), tray (n = 18), basket (n = 18) and postharvest equipments knife (n = 18), cutting board (n = 15), packing material (n = 15) and glove (n = 18), were collected twice from three radish sprout facilities located in Gyeonggi-do, Republic of Korea, during April–May and August 2016. The radish sprouts were cultivated hydroponically in all the farms. The farms A and B used ground water and irrigation water for radish sprout cultivation while farm C used stream water along with ground water for irrigation without filtration or disinfection.

Surfaces of nutrients storage tank, fabric, tray, basket, knife, cutting board and packing material were swabbed with commercial kit, and glove samples were collected using the glove-juice method [14]. Seeds and radish sprouts (200 geach) from the cultivation and postharvest steps were collected and placed in sterile plastic bags. For hydroponic farming, the farms diluted nutrient solutions A and B with water and delivered them through irrigation water to the sprouts two days post germination. Two-liter water and nutrient solutions were individually collected in sterile bottles.

Analysis of coliform and *Escherichia coli*

For the quantitative analysis of coliforms and *E. coli*, 25 g of seed and sprout samples were placed in 225 ml of 0.1% peptone water (PW) (Oxoid, Basingstoke, UK) and homogenized in a BagMixer® (Interscience, Saint-Nom-la-Bretéche, France). Surface samples were homogenized using a vortex prior to dilution. These pretreated samples were serially diluted 1:10 in 0.1% PW and 1 ml aliquots were inoculated on 3 M™ Petrifilm™ *E. coli*/coliform count plates (3 M Microbiology, Minnesota, USA), followed by incubation at 37 °C for 24–48 h. Water samples were evaluated for coliforms and *E. coli* using the Colilert-18 detection kit from IDEXX laboratories (Westbrook, Maine, USA).

For the qualitative analysis of *E. coli* and *E. coli* O157:H7, 25 g of seed and sprouts were placed in 225 ml of *E. coli* broth (EC) (Oxoid) and modified tryptone soy broth (mTSB) (Oxoid) for *E. coli* and *E. coli* O157:H7 detection, respectively, and homogenized in a BagMixer®. For the swab and glove samples, 1 ml of homogenate was dispensed into 9 ml of EC broth and mTSB individually. Water samples (250 ml) were filtered using a 0.45 µm membrane filter (Merck-Millipore, Carrigtwohill, Ireland). Each filter was placed in sterile bottles containing 40 ml of EC broth or mTSB and incubated at 44 °C and 37 °C for 24 h, respectively. The enriched cultures were streaked onto eosin-methylene blue agar plates (Oxoid) and CHROMagar™ *E. coli* O157 (CHROMagar™, Paris, France) plates for *E. coli* and *E. coli* O157:H7 using a disposable loop. After incubation at 37 °C for 24 h, typical colonies were picked and identified using the VITEK 2 system, an automated system that performs microbial identification and antibiotic susceptibility testing (bioMérieux-Vitek, USA). Additionally, the *E. coli* isolates were further identified and classified by 16 s rRNA sequencing (Macrogen, Seoul, Korea) [15].

Serotyping and virulence determination

Colonies of *E. coli* and *E. coli* O157:H7 were serotyped using *Escherichia coli* O-antigen antisera kit (Joong Kyeom, Republic of Korea). Genomic DNA was extracted from the isolates and retention of virulence determinants (*eaeA, bfpA, ipaH, aggR, vt1, vt2, lt, st*) in the isolates were determined by PCR using the PowerChek™ Diarrheal *E. coli* 4-plex PCR Detection Premix I/Premix II kit (KogeneBioTech Co., Ltd., Republic of Korea) [16].

Multi locus sequence typing (MLST)

To determine clonal relationship of the *E. coli* isolates, genomic DNA of the isolates was prepared and the housekeeping genes (Table 3) were amplified by PCR. The resulting DNA fragments were purified and sequenced

(GnCBio Co. Ltd., Republic of Korea). Sequence types (STs) of the *E. coli* isolates were assigned using the Bacterial Isolate Genome Sequence Database (BIGSdb) tool from the PubMLST server.

Cytotoxicity assay

The nematode *Caenorhabditis elegans* killing assay was carried out to evaluate the pathogenicity of *E. coli* isolates in compliance with Lee's method with modifications [17]. Overnight cultures of *E. coli* isolates possessing pathogenic genes (2×10^9 CFU/ml) grown in Luria–Bertani broth at 37 °C for 18 h were spread on nematode growth medium (NGM). The nematodes were synchronized by hypochlorite bleaching and subsequently cultured on NGM in the presence of *E. coli*. Repeatedly, synchronized L1 larvae were transferred to a new plate containing *E. coli* OP50, allowing the production of L4 larvae serially. After three days, 20 L4/young adult hermaphrodites were placed on NGM, incubated at 25 °C for 45 min, and transferred to a new plate every single day. The survival of *C. elegans* without the presence of progeny and L4 nematodes was examined using the Kaplan–Meier method, and differences were determined with log-rank test.

Results and discussion

Assessment of coliforms and *E. coli* contamination in radish seed sprouts production units

The levels of coliforms and *E. coli* in the samples from sprouts, farming tools, and water from farms A, B, and C are presented in Tables 1 and 2. Of the 324 samples collected during spring and summer, the levels of coliforms were higher (> 3.75 log CFU/g) in radish sprouts in summer than in spring. Coliforms were more prevalent in tray (4.96 ± 1.44), cutting board (5.62 ± 0.59) and glove (4.30 ± 0.00) from farm A and basket (5.57 ± 0.49), cutting board (5.40 ± 0.93) and glove (4.82 ± 0.11) from B during summer season compared to all the other samples including water (< 2 log CFU/ml), agricultural materials (< 3 log CFU/ml). Corresponding to our findings, several studies have reported increased microbial levels in postharvest sprouts than in seeds [18, 20]. The incidence of *E. coli* was significantly higher in the preharvest (50%) and postharvest (39%) sprouts and water samples (33%) from farms B and C. *E. coli* was detected in the basket (5%), glove (16%), and knife (11%) samples, while no *E. coli* were detected in the other samples.

Seeds usually acquire 10^2 to 10^3 CFU/g of coliforms from the surrounding environment [21]. These bacterial populations can significantly increase during the sprouting process to as high as 10^8 to 10^{11} CFU/g [22, 23] without damaging the appearance of the final product [24]. Unaware of the contaminated seeds (0.90 ± 0.85 log CFU/g), farms B and C used the seeds for cultivation during the summer season, which resulted in a large number of coliforms in the pre- and post-harvest radish sprouts (3.75–4.92 log CFU/g).

Water can potentially cross-contaminate sprouts by transferring microbes from other sources during production and processing [25]. In this study, the seeds used for hydroponic germination in farms A and C during spring and summer were free of *E. coli* contamination. However, *E. coli* was detected in the preharvest and postharvest radish sprouts and water including ground water, irrigation water and stream water stored in container. Since water was the only source that was in contact with the seeds during sprouting, it is coherent that water transmitted *E. coli* to vulnerable seeds during irrigation and contaminated the sprouts. Even though knives and cutting boards were washed with water by the farms, they can still be contaminated during processing by transmitting *E. coli* from contaminated to non-contaminated fresh produce [26]. For instance, in this study, no coliforms were detected in the seeds and preharvest radish sprouts of farm A during the spring season, but during harvesting, the coliforms were transferred to the postharvest radish sprouts via contaminated knives (3.69 ± 0.03 log CFU/g) and gloves (2.84 ± 2.46 log CFU/g) used by the workers.

Molecular characterization and virulence determination of *E. coli* isolates

The *E. coli* isolates were discriminated into virulent and avirulent strains by identifying the presence of pathogenic genes in their genome. Of the 48-suspected *E. coli* isolates, only eight isolates from farms B and C were identified as pathogenic based on the results of serotyping and virulence determination tests (Table 3). Eight isolates belonged to the O-serogroup, five isolates and one isolate from water were assigned as serotypes O110 and O71, respectively, and the other two isolates were nontypeable O-antigens (ONT). Pathogenic *E. coli* possess certain combinations of virulence genes that cause food poisoning [27]. Based on the combination of virulence genes, *E. coli* strains can be classified as enteropathogenic (EPEC), enterotoxigenic (ETEC), enteroinvasive (EIEC), enteroaggregative (EAEC), and enterohemorrhagic (EHEC) [28]. The O110 and ONT isolates possessed the virulence gene *eaeA* (*E. coli* attaching and effacing), while the O71 isolate contained the *ipaH* (invasion plasmid antigen H) gene, which designates these isolates as EPEC O110, EPEC ONT, and ETEC O71 (Table 3).

Escherichia coli strains with serotype O110 were previously isolated from various sources in different countries, including healthy adults in Japan [29], dairy samples in France [30], and ovine strains in Spain [31]. However, all

Table 1 Evaluating the frequency of coliforms (log CFU/g) on radish seed sprouts and their production environment

Source		No. of samples	Sample	A		B		C		Total
				Spring	Summer	Spring	Summer	Spring	Summer	
Seeds and Sprouts		18	Seeds	ND	ND	ND	0.90 ± 0.85	ND	0.49 ± 0.85	0.23 ± 0.38
		18	2nd Day sprouts	ND	1.54 ± 0.21	ND	4.92 ± 0.51	ND	3.75 ± 0.05	1.7 ± 2.15
		18	4th Day sprouts	ND	ND	ND	4.83 ± 0.02	ND	4.08 ± 0.25	1.49 ± 2.31
		18	6th Day sprouts	ND	ND	ND	4.77 ± 0.11	ND	4.60 ± 0.10	1.56 ± 2.42
		18	Packaged sprout	4.65 ± 0.24	ND	ND	4.21 ± 0.34	ND	3.99 ± 0.09	2.14 ± 2.36
Water		18	Ground water	ND	0.67 ± 1.15	ND	ND	ND	ND	0.11 ± 0.27
		18	Irrigation water	ND	ND	ND	ND	0.98 ± 1.71	ND	0.16 ± 0.4
		6	Stream water stored in container	NS	NS	NS	NS	1.09 ± 1.88	ND	0.18 ± 0.44
Agricultural materials		18	Nutrient solution A	ND	ND	1.37 ± 2.38	ND	ND	ND	0.22 ± 0.56
		18	Nutrient solution B	ND	ND	ND	ND	ND	ND	0
		18	Diluted nutrient solution A + B	ND	3.08 ± 0.07	ND	ND	ND	ND	0.51 ± 1.26
Equipment	Cultivation	18	Nutrient solution storage tank	ND	ND	ND	0.77 ± 1.33	ND	ND	0.13 ± 0.31
		18	Fabric	ND	1.23 ± 2.14	ND	2.00 ± 0.00	ND	ND	0.54 ± 0.87
		18	Tray	0.40 ± 0.70	4.96 ± 1.44	ND	4.34 ± 0.35	0.22 ± 0.38	ND	1.65 ± 2.33
		18	Basket	ND	1.70 ± 3.10	ND	5.57 ± 0.49	ND	0.97 ± 0.85	1.37 ± 2.17
	Post-harvest	18	Knife	3.69 ± 0.03	3.48 ± 0.26	3.07 ± 2.73	3.86 ± 0.49	3.59 ± 0.57	0.67 ± 1.15	3.06 ± 1.2
		15	Cutting board	NS	5.62 ± 0.59	ND	5.40 ± 0.93	3.91 ± 0.19	ND	2.49 ± 2.79
		15	Packing material	NS	1.49 ± 1.31	ND	1.60 ± 2.78	0.23 ± 0.41	ND	0.55 ± 0.77
		18	Glove	2.84 ± 2.46	4.30 ± 0.00	ND	4.82 ± 0.11	ND	4.12 ± 0.29	2.68 ± 2.18
			Total	0.61 ± 1.42	1.48 ± 1.89	0.23 ± 0.76	2.53 ± 2.26	0.53 ± 1.18	1.19 ± 1.82	1.09 ± 0.99

* All values shown in this table are the mean ± standard deviation of three independent experiments. *The values for each sample mentioned in this table are the average of three replicates. **ND* not detected, **NS* not sampled

Table 2 Evaluating the frequency of *Escherichia coli* detection on radish seed sprouts and their production environment

Source		No. of samples	Sample	A		B		C		Total
				Spring	Summer	Spring	Summer	Spring	Summer	
Seeds and Sprouts		18	Seeds	0	0	0	3/3 (100%)	0	0	3/18 (16%)
		18	2nd Day sprouts	0	1/3 (33%)	1/3 (33%)	2/3 (66%)	3/3 (100%)	1/3 (33%)	8/18 (44%)
		18	4th Day sprouts	0	0	3/3 (100%)	1/3 (33%)	3/3 (100%)	2/3 (66%)	9/18 (50%)
		18	6th Day sprouts	0	0	0	0	3/3 (100%)	0	3/18 (16%)
		18	Packaged sprout	0	1/3 (33%)	1/3 (33%)	2/3 (66%)	0	3/3 (100%)	7/18 (39%)
Water		18	Ground water	0	0	0	0	3/3 (100%)	3/3 (100%)	6/18 (33%)
		18	Irrigation water	0	1/3 (33%)	1/3 (33%)	0	1/3 (33%)	0	3/18 (16%)
		6	Stream water stored in container	NS	NS	NS	NS	3/3 (100%)	0	3/6 (50%)
Agricultural Materials		18	Nutrient solution A	0	0	0	0	0	0	0/18 (0%)
		18	Nutrient solution B	0	0	0	0	0	0	0/18 (0%)
		18	Diluted nutrient solution A + B	0	0	0	0	0	0	0/18 (0%)
Equipment	Cultivation	18	Nutrient solution storage tank	0	0	0	0	0	0	0/18 (0%)
		18	Fabric	0	0	0	0	0	0	0/18 (0%)
		18	Tray	0	0	0	0	0	0	0/18 (0%)
		18	Basket	0	0	0	0	1/3 (33%)	0	1/18 (5%)
	Post-harvest	18	Knife	0	2/3 (66%)	0	0	0	0	2/18 (11%)
		15	Cutting board	NS	0	0	0	0	0	0/15 (0%)
		15	Packing material	NS	0	0	0	0	0	0/15 (0%)
		18	Glove	0	0	0	0	0	3/3 (100%)	3/18 (16%)
			Total	0	5/57 (9%)	6/57 (10%)	8/57 (14%)	17/57 (30%)	12/57 (21%)	48/324 (14%)

Total—number of *E. coli* positive / number of tested samples. *The values for each sample mentioned in this table are the average of three replicates. **NS* not sampled

Table 3 Serological and genotypic characterization of *Escherichia coli* isolates

E. coli isolate	Farm	Season	Source	No. of isolates	Virulence genes		*E. coli* allelic profile (MLST)								ST
					eaeA	*ipaH*	*dinB*	*icdA*	*pabB*	*polB*	*putP*	*trpA*	*trpB*	*uidA*	
EPEC O110	C	Spring	Container water	5	+	-	1	23	11	9	2	144	46	137	New1
EPEC ONT	C	Spring	Container water	2	+	-	1	23	11	9	2	144	46	137	New2
ETEC O71	B	Summer	Irrigation water	1	-	+	11	35	4	52	125	146	141	2	New3

* The numbers in the MLST column represent the number of alleles per locus; *ST* sequence types

the O110 isolates possessed either one of the Shiga-toxin genes (*stx1* and *stx2*), but none were *eaeA*-positive. The *eaeA* gene codes for the surface protein Intimin, a virulence factor usually found in EPEC (O127:H6) and EHEC (O157:H7) strains [32]. Intimins localize to the outer membrane of *E. coli* cells and promote intimate bacterial adhesion to the intestinal epithelia, resulting in the formation of effacing lesions that are necessary for causing EPEC and EHEC diarrhea [33]. The ETEC O71 serotype strains were isolated from patients with enteric illness in Brazil [34] and Vietnam [35]. The similarity between the strains was the presence of the *eaeA* gene; however, the strain isolated in our study was *eaeA*-negative and *ipaH*-positive (Table 3). The *ipaH* gene is a type 3 effector that facilitates the penetration of pathogens into intestinal cells [36]. The MLST database revealed three different STs that did not exactly match with other STs in the database (Table 3), indicating that the isolates could be new subtypes.

Evaluation of cytotoxicity of *E. coli* isolates using *Caenorhabditis elegans*

The cytotoxicity of the *E. coli* isolates was determined by comparing the survival rate of isolate-exposed *C. elegans* with that of those exposed to *E. coli* OP50. The survival rate of those exposed to the *E. coli* isolate EPEC O110 was significantly reduced from day 8 compared to *E. coli* OP50, which showed a reduction on day 12, indicating that the isolate EPEC O110 is pathogenic (Fig. 1). The isolates EPEC ONT and ETEC O71 caused survival rates similar to those of *E. coli* OP50, indicating that the isolates were non-pathogenic. Merkx-Jacques et al. (2013) demonstrated that the *E. coli* isolates that were predicted to be pathogenic by genotyping assays were able to kill *C. elegans* [37]. Correspondingly, in our study, only one of the eight isolates was a toxin producing *E. coli*.

Conclusion

The aim of this study was to identify the source of *E. coli* contamination in commercial radish production units and emphasize the need to minimize contamination by following good agricultural practices during sprout production and good hygienic practices during processing, packaging, and shipping. Genotyping and toxicity studies revealed that the pathogenic *E. coli* strain EPEC O110 was more prevalent in the stream water stored in the containers from farm C. The contaminated water may have potentially transferred pathogenic *E. coli* to the germinated seeds and sprouts during production and processing. In addition, the knives, cutting boards, trays and

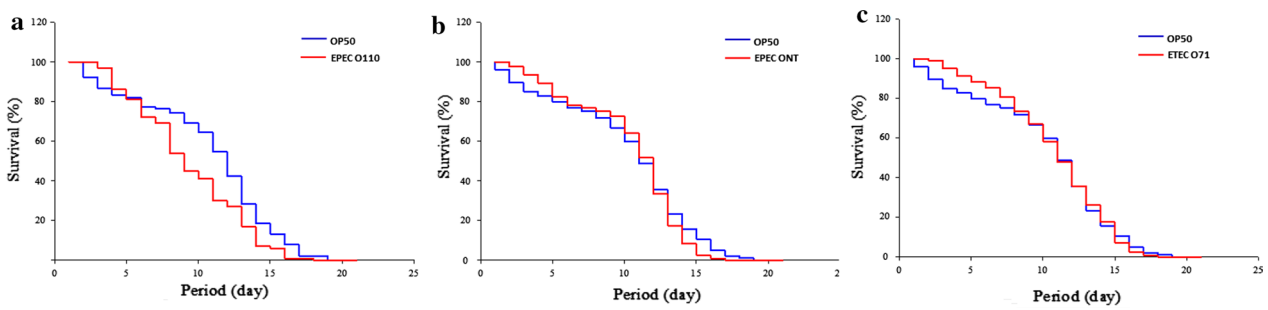

Fig. 1 Kaplan–Meier survival plots of *Caenorhabditis elegans* infected with *Escherichia coli* isolates. The nematode worms grown in nematode growth medium were fed with three different *E. coli* isolates (EPEC 0110, EPEC ONT and ETEC O71) along with control *E. coli* OP50 and incubated at 25 °C. The mortality of the worms was monitored every day and the data were generated using the Kaplan–Meier method. Blue line indicates the survival of nematodes fed with *E. coli* OP50 while the red line indicates the mortality of the worms fed with *E. coli* isolates **a** EPEC 0110, **b** EPEC ONT, and **c** ETEC O71

gloves used during the processing of sprouts may have transmitted the pathogens through cross-contamination. The prevalence of diarrhea causing *E. coli* in the water that is stored in the container in radish sprout production units accentuates the need to sanitize stream water before irrigation and routinely clean the containers and other equipments at regular intervals.

Authors' contributions
NR and JHY contributed to the writing of the manuscript and performed the majority of data analysis. BY, NBH, WK, HK performed experiments and prepared raw materials. BYP contributed to the discussion of experimental results. SRK planned and led this research. All authors read and approved the final manuscript.

Funding
This study was funded by the National Institution of Agricultural Science, Rural Development Administration, Republic of Korea.

Availability of data and materials
All the data analyzed during this study are included in this article.

Competing interests
The authors declare that they have no competing interests.

Author details
[1] Rural Development Administration, Microbial Safety Team, National Institute of Agricultural Sciences, Wanju 55365, Republic of Korea. [2] Department of Animal Science and Institute of Milk Genomics, Chonbuk National University, Jeonju 54896, Korea. [3] Crop Foundation Division, National Institute of Crop Sciences (NICS), Rural Development Administration (RDA), Wanju 55365, Republic of Korea. [4] Research Policy Bureau, Research Policy Division, Rural Development Administration (RDA), Jeonju 54875, Republic of Korea. [5] Laboratory of Enzyme and Protein Technology, Institute of Biotechnology, Hue University, Hue 530000, Vietnam. [6] Agro-Food Safety & Crop Protection Department, National Institution of Agricultural Science, Rural Development Administration, Wanju 55365, South Korea.

Received: 23 November 2020 Accepted: 5 February 2021
Published online: 08 March 2021

References

1. Yuan G, Wang X, Guo R, Wang Q (2010) Effect of salt stress on phenolic compounds, glucosinolates, myrosinase and antioxidant activity in radish sprouts. Food Chem 121(4):1014–1019
2. Neetoo H, Chen H (2010) Pre-soaking of seeds enhances pressure inactivation of *E. coli* O157: H7 and Salmonella spp. on crimson clover, red clover, radish and broccoli seeds. Int J Food Microbiol. 137(2–3):274–280
3. Kim Y, Kim H, Song M, Rhee MS (2015) Decontamination method using heat and relative humidity for radish seeds achieves a 7-log reduction of *Escherichia coli* O157: H7 without affecting product quality. Int J Food Microbiol. 201:42–46
4. Kintz E, Byrne L, Jenkins C, McCARTHY N, Vivancos R, Hunter P (2019) Outbreaks of shiga toxin-producing *escherichia coli* linked to sprouted seeds, salad, and leafy greens: a systematic review. J Food Prot 82(11):1950–1958
5. Michino H, Araki K, Minami S, Takaya S, Sakai N, Miyazaki M et al (1999) Massive Outbreak of *Escherichia coli* O157: H7 Infection In Schoolchildren in Sakai City, Japan, Associated with Consumption of White Radish Sprouts. Am J Epidemiol 150(8):787–796
6. Karch H, Denamur E, Dobrindt U, Finlay BB, Hengge R, Johannes L et al (2012) The enemy within us: lessons from the 2011 European *Escherichia coli* O104: H4 outbreak. EMBO Molecular Med 4(9):841–848
7. Centers for Disease Control and Prevention. Multistate Outbreak of Shiga Toxin-producing *Escherichia coli* O26 Infections Linked to Raw Clover Sprouts at Jimmy John's Restaurants 2012 [https://www.cdc.gov/ecoli/2012/o26-02-12/index.html].
8. Centers for Disease Control and Prevention. Outbreak of *E. coli* Infections Linked to Clover Sprouts 2020 [https://www.cdc.gov/ecoli/2020/o103h2-02-20/index.html].
9. Sagoo S, Little C, Ward L, Gillespie I, Mitchell RT (2003) Microbiological study of ready-to-eat salad vegetables from retail establishments uncovers a national outbreak of salmonellosis. J Food Prot 66(3):403–409
10. Pachepsky Y, Shelton DR, McLain JE, Patel J, Mandrell RE. Irrigation waters as a source of pathogenic microorganisms in produce: a review. Advances in Agronomy. 113: Elsevier; 2011. p. 75–141.
11. Benincasa P, Falcinelli B, Lutts S, Stagnari F, Galieni A (2019) Sprouted Grains: A Comprehensive Review. Nutrients 11(2):421
12. Machado-Moreira B, Richards K, Brennan F, Abram F, Burgess CM (2019) Microbial Contamination of Fresh Produce: What, Where, and How? Comprehensive Rev Food Sci Food Safety 18(6):1727–1750
13. Brandt MJ, Johnson KM, Elphinston AJ, Ratnayaka DD (2017) Chapter 7 - Chemistry, Microbiology and Biology of Water. In: Brandt MJ, Johnson KM, Elphinston AJ, Ratnayaka DD (eds) Twort's Water Supply, 7th edn. Butterworth-Heinemann, Boston, pp 235–321
14. Yoon Y, Kim S-R, Kang D-H, Shim W-B, Seo E, Chung D-H (2008) Microbial Assessment in School Foodservices and Recommendations for Food Safety Improvement 73(6):M304–M313
15. Perveen N, Muzaffar SB, Vijayan R, Al-Deeb MA (2020) Microbial communities associated with the camel tick, Hyalomma dromedarii: 16S rRNA gene-based analysis. Scientific Reports 10(1):17035
16. Yun B, Kim Y, Hung NB, Oh K-H, Kim W-I, Ham H et al (2018) Microbiological quality and characteristics of isolated Escherichia coli in irrigation water used in Napa cabbage cultivation. Applied Biological Chemistry 61(5):567–574
17. Lee S-J, Kenyon C (2009) Regulation of the longevity response to temperature by thermosensory neurons in *Caenorhabditis elegans*. Curr Biol 19(9):715–722
18. Fu T-J, Reineke KF, Chirtel S, Vanpelt OM (2008) Factors influencing the growth of *Salmonella* during sprouting of naturally contaminated alfalfa seeds. J Food Prot 71(5):888–896
19. Yang Y, Meier F, Ann Lo J, Yuan W, Lee Pei Sze V, Chung HJ et al (2013) Overview of recent events in the microbiological safety of sprouts and new intervention technologies. Comprehensive Rev Food Sci Food Safety. 12(3):265–280
20. Landry KS, Sela DA, McLandsborough L (2018) Influence of sprouting environment on the microbiota of sprouts. J Food Saf 38(1):e12380
21. Feng P (1997) A summary of background information and foodborne illness associated with the consumption of sprouts. Center for Food Safety and Applied Nutrition, Washington, DC, pp 96–99
22. Gandhi M, Matthews KR (2003) Efficacy of chlorine and calcinated calcium treatment of alfalfa seeds and sprouts to eliminate Salmonella. Int J Food Microbiol 87(3):301–306
23. Peñas E, Gómez R, Frías J, Vidal-Valverde C (2008) Application of high-pressure treatment on alfalfa (*Medicago sativa*) and mung bean (*Vigna radiata*) seeds to enhance the microbiological safety of their sprouts. Food Control 19(7):698–705
24. Taormina PJ, Beuchat LR, Slutsker L (1999) Infections associated with eating seed sprouts: an international concern. Emerg Infect Dis 5(5):626
25. Decol LT, Casarin LS, Hessel CT, Batista ACF, Allende A, Tondo EC (2017) Microbial quality of irrigation water used in leafy green production in Southern Brazil and its relationship with produce safety. Food Microbiol 65:105–113
26. Yang Y, Luo Y, Millner P, Turner E, Feng H (2012) Assessment of *Escherichia coli* O157: H7 transference from soil to iceberg lettuce via a contaminated field coring harvesting knife. Int J Food Microbiol 153(3):345–350
27. Grauke LJ, Kudva IT, Yoon JW, Hunt CW, Williams CJ, Hovde CJ (2002) Gastrointestinal tract location of *Escherichia coli* O157: H7 in ruminants. Appl Environ Microbiol 68(5):2269–2277
28. Chapman TA, Wu X-Y, Barchia I, Bettelheim KA, Driesen S, Trott D et al (2006) Comparison of virulence gene profiles of *Escherichia coli* strains isolated from healthy and diarrheic swine. Appl Environ Microbiol 72(7):4782–4795
29. Morita-Ishihara T, Iyoda S, Iguchi A, Ohnishi M (2016) Secondary Shiga Toxin-Producing *Escherichia coli* Infection, Japan, 2010–2012. Emerg Infect Dis 22(12):2181

30. Pradel N, Bertin Y, Martin C, Livrelli V (2008) Molecular analysis of shiga toxin-producing *Escherichia coli* strains isolated from hemolytic-uremic syndrome patients and dairy samples in France. Appl Environ Microbiol 74(7):2118–2128
31. Blanco M, Blanco JE, Mora A, Rey J, Alonso JM, Hermoso M, Hermoso J, Alonso MP, Dahbi G, González EA, Bernárdez MI (2003) Serotypes, virulence genes, and intimin types of Shiga toxin (verotoxin)-producing *Escherichia coli* isolates from healthy sheep in Spain. J Clin Microbiol 41(4):1351–1356
32. Adams TM, Schmoldt H-U, Kolmar H (2004) FACS screening of combinatorial peptide and protein librariers displayed on the surface of *Escherichia coli* cells. Evolutionary Methods in Biotechnology: Clever Tricks for Directed Evolution 1:31–46
33. Jerse AE, Yu J, Tall BD, Kaper JB (1990) A genetic locus of enteropathogenic *Escherichia coli* necessary for the production of attaching and effacing lesions on tissue culture cells. Proc Natl Acad Sci 87(20):7839–7843
34. Ori EL, Takagi EH, Andrade TS, Miguel BT, Cergole-Novella MC, Guth BE, Hernandes RT, Dias RC, Pinheiro SR, Camargo CH, Romero EC. Diarrhoeagenic *Escherichia coli* and *Escherichia albertii* in Brazil: pathotypes and serotypes over a 6-year period of surveillance. Epidemiology & Infection. 2019;147.
35. Hien BT, Scheutz F, Cam PD, Serichantalergs O, Huong TT, Thu TM, Dalsgaard A (2008) Diarrheagenic *Escherichia coli* and Shigella strains isolated from children in a hospital case-control study in Hanoi. Vietnam J Clin Microbiol 46(3):996–1004
36. Das A, Mandal J (2019) Extensive inter-strain diversity among clinical isolates of Shigella flexneri with reference to its serotype, virulence traits and plasmid incompatibility types, a study from south India over a 6-year period. Gut Pathogens 11(1):33
37. Merkx-Jacques A, Coors A, Brousseau R, Masson L, Mazza A, Tien YC, Topp E (2013) Evaluating the pathogenic potential of environmental *Escherichia coli* by using the *Caenorhabditis elegans* infection model. Appl Environ Microbiol 79(7):2435–2445

Publisher's Note