Evaluation of the microbial contamination of fresh produces and their cultivation environments from farms in Korea

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Abstract In this study, a total of 195 samples including fresh produce and farming environments was used to perform the microbial risk assessment. Levels of total aerobic bacteria ranged from 2.77 to 5.99, 6.28 to 7.81, and 1.31 to 2.74 log₁₀ CFU/g, whereas levels of coliforms were ≤ 2.48, ≤ 3.35, and ≤ 0.85 log₁₀ CFU/g, levels of *Escherichia coli* were ≤ 1.04, ≤ 0.12, and ≤ 1.69 log₁₀ CFU/g in fresh produce, soil, and irrigation water, respectively. When the presence of pathogenic bacteria was detected, only *Bacillus cereus* and *Staphylococcus aureus* were isolated from 14 (7.2%) and 7 (3.6%) samples out of 195 samples, respectively. From the results, it was difficult to find a strong correlation between microbial contamination of fresh produce and their farming environments. However, continuous monitoring of agricultural products

and related environments should be undertaken in order to ensure the microbial safety of fresh produce.

Keywords Microbiological quality · Pathogens · Fresh produce · Irrigation water · Soil

Introduction

Fresh fruits and vegetables are very popular since these products contain various essential nutrients, including vitamins, minerals, and fibers (Maffei et al., 2013). In spite of the health benefits, foodborne disease outbreaks associated with the consumption of fresh produce have been continuously reported and increased frequencies in recent

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years (Delaquis et al., 2007; MFDS, 2018). In general, fresh fruits and vegetables are eaten raw or without minimal-thermal processing, thereby posing a risk of potential foodborne outbreaks (Stine et al., 2005). Microbial contamination of fresh produce can occur in all kinds of agricultural environments where fruits and vegetables (or crops) grow from harvest till practical consumption (Beuchat, 2002). Potential pre-harvest contamination sources include soil, irrigation water, farm animal feces, and human handling (Holvoet et al., 2014; Ibenyassine et al., 2006). The use of irrigation water or soil provides important nutrients for crops, but improperly treated irrigation water or soil can contain foodborne pathogens (Steele and Odumeru, 2004). The adoption of the most suitable practices of agricultural management is essential in order to improve the safety of produce and preserve irrigation water or soil quality (Selma et al., 2007). Additionally, all of these factors can influence the survival and growth of human pathogens (Thunberg et al., 2002). Major foodborne pathogens closely linked to fresh produce include *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella enterica* serovar Typhimurium, *Staphylococcus aureus*, *Bacillus cereus*, and *Clostridium* spp. (Bennett et al., 2013; Loir et al., 2003; Loncarevic et al., 2005). In particular, *E. coli* O157:H7 and *Salmonella* spp. may exist in the gastro-intestinal tract of animals, as well as in animal fecal matter used as fertilizer (Loncarevic et al., 2005). It has been well documented that strains of *L. monocytogenes* are commonly found in soil, animal fecal matter, and contaminate vegetables growing in the field (Thunberg et al., 2002). *B. cereus* contamination has been associated with soil, cereal, grain, and dairy equipment (Bartoszewicz et al., 2008). In addition, counts of fecal coliforms and *E. coli* are the microbial indicators most commonly used by water quality guidelines to dictate irrigation water quality (Steele and Odumeru, 2004). As a result, fecal coliform and *E. coli* counts are now considered an ideal measure of fecal contamination of water (Barrell et al., 2000).

The purpose of this study was therefore to assess the foodborne pathogenic microbial prevalence on fresh produce and agricultural farming fields, including irrigation water and soil from agricultural farms located in Chungcheongnam-do, Gyeongsang-do, Jeolla-do, Kanwon-do, and Kyunggi-do in the Republic of Korea from 2016 to 2017.

Materials and methods

Sampling

A total of 195 samples including fresh produce (Chinese cabbage, romaine lettuce, cucumber, pepper, tomato, and strawberry) and farming environments (irrigation water and

soil) were randomly collected from agricultural farms located in Chungcheongnam-do, Gyeongsang-do, Jeonl-do, Kanwon-do, and Kyunggi-do in the Republic of Korea from 2016 to 2017. Fresh produce was collected directly from the farm fields and were transferred into sterile zip-lock bags without being washed. Gloves were sanitized with alcohol swabs between collections to prevent cross-contamination. The collected samples were Chinese cabbage, romaine lettuce, cucumber, pepper, tomato, and strawberry. Correspondingly irrigation water was collected in 1 L sterile bottles. Additionally, soil was collected from the same locations where the fresh produce were sampled using 50 mL conical centrifuge tube. All samples were transported to the laboratory immediately after sampling on ice in cardboard boxes. The samples were stored at 4 °C after their arrival from farms and used within 24 h.

Quantitative analysis of microbiological contamination

Twenty-five grams of produce (Chinese cabbage, romaine lettuce, cucumber, pepper, tomato, and strawberry) were homogenized in 125 mL (1:5) sterile water containing 0.2% peptone (PW, Difco) using sterile filter stomacher bags (Difco Laboratories, Detroit, MI, USA) and a stomacher (BagMixer 400, Interscience, Breteche, France) for 1 min. Chinese cabbage leaves were separated into the outer and inner leaves. The first three layers of the Chinese cabbage were collected as outer leaves, while the yellow leaves were collected as the inner leaves. In addition, each 10 g of soil sample was homogenized in 100 mL (1:10) sterile water containing 0.2% peptone (Difco) and massaged thoroughly by hand for 1 min. TAB, Coliform, *E. coli*, and Y/M were enumerated by the petrifilm aerobic count plate, petrifilm *E. coli*/coliform count plate, and petrifilm yeast/molds count plate (3 M, Seoul, Korea), respectively. 1.0 mL diluted sample (diluted with 0.2% peptone) or water samples was added to the center of a film base, and a top film was carefully placed down on the inoculums. The sample was distributed over a prescribed growth area using a downward pressure at the center of a plastic spreader. The plates were left undisturbed for 1 min to permit the gel to solidify prior to incubation at 37 °C for 24 h for TAB, Coliform, and *E. coli*, and 30 °C for 24–48 h for Y/M. For TAB, the colonies in the countable range (30–300 colonies) were enumerated. Red colonies with gas bubbles were counted as coliforms, and blue colonies with gas were considered as *E. coli*. Additionally, pink-tan to blue-green colonies were counted as yeasts, and diffuse edges colonies were counted as molds. For *B. cereus*, five 0.2 mL aliquots of the serially diluted solutions for fresh produce and soil were inoculated onto five Mannitol Egg Yolk Polymyxin Agar plate (MYP, Difco)

and incubated at 30 °C for 24 h. Pink-colored colonies with white areas were counted as *B. cereus*.

Analysis and identification of pathogens

The microbiological analytical methods were used in this study followed the Korean Food Code or other standard methods (MFDS, 2016). The prevalence of *B. cereus*, *L. monocytogenes*, *Salmonella* spp., *S. aureus*, *E. coli* O157:H7, and *C. perfringens* in the produce, soil, and irrigation water samples was determined. Each 25 g of produce, used for the detection of six different pathogens, was diluted with 100 mL of enrichment culture broths and homogenized for 1 min (Table 1). In addition, each 10 g of soil samples was mixed with 90 mL of enrichment culture broths and massaged thoroughly by hand for 1 min. Microbial quality of irrigation water samples was analyzed by filtering 100 mL of water through sterile 0.45 µm pore size membrane filters (Fisher brand Water Testing Membrane Filters, Thermo Fisher Scientific, Pittsburgh, PA), and the filters were placed on the plates with appropriate selective media. The prevalence of *B. cereus* in the samples collected for the quantitative assays described above was determined. Plate of MYP (Difco) were inoculated from diluted samples (diluted with 0.2% peptone) for fresh produce and soil, while the water-filtered membrane paper was placed on the selective media and incubated at 30 °C for 24 h. The presumptive colonies were confirmed using API 50 CHB kit (BioMeriux, Marcy l'Etoile, France). Each of bacteria presumably recognized as a pathogen was further identified by 16 rRNA sequencing. Its identified colonies were counted and calculated by multiplying the dilution factor. For *L. monocytogenes*, the pre-enriched sample was streaked on modified Oxford Agar plate (OAB, Difco), and a filter was placed on the medium. After incubation, the presumptive colonies were confirmed using the Latex agglutination test kit (*Listeria* Diagnostic Reagents test kit, Oxoid, Hampshire, UK). The pathogen was further identified by 16s rRNA sequencing. For *Salmonella* spp., 0.1 mL of pre-enriched sample was transferred to 10 mL Rappaport–Vassiliadis R10 (RV) medium (RV, Difco) and another 1 mL mixture to 10 mL Tetrathionate Broth (TB, Difco). Subsequently, the filter was placed on the medium. RV medium was incubated at 42 °C for 24 h and TB medium at 37 °C for 24 h. The enriched media were streaked on Xylose Lysine Desoxycholate (XLD, Oxoid, Basingstoke, Hampshire, UK) and incubated at 37 °C for 24 h. The presumptive colonies were confirmed by API 20 E (BioMeriux). Each bacterium presumably recognized as a pathogen was further identified by 16s rRNA sequencing. For *S. aureus*, the pre-enriched sample was streaked on a Baird Parker plate (BPA, Difco), and a filter was placed on the medium. After

incubation, the presence of *S. aureus* was assumed by round, black convex colonies with a clear zone surrounding the colony. The presumptive colonies were confirmed using the API Staph kit (BioMeriux). Each bacterium presumably recognized as a pathogen was further identified by 16s rRNA sequencing. For *E. coli* O157:H7, the pre-enriched sample was streaked on sorbitol MacConkey agar (SMAC, Difco), and a filter was placed on the medium. Typical lactose-fermenting colonies were confirmed using the *E. coli* O157 Diagnostic Reagents test kit (Oxoid). The pathogen was further identified by 16s rRNA sequencing. For *C. perfringens*, 0.1 mL of pre-enriched sample was inoculated in 10 mL Cooked meat medium (Oxoid) and incubated at 37 °C for 24 h under anaerobic conditions. The enriched broth was streaked on a *Perfringens* agar base (TSC, Difco). After anaerobic incubation, the presence of *C. perfringens* was confirmed by the appearance of a black colony. The presumptive colonies were identified by 16s rRNA sequencing.

Results and discussion

Levels of microbial contamination

Microbial levels of fresh produce, soil, and irrigation water are shown in Table 2 and Fig. 1. Table 2 showed that a total of 195 samples were collected during July 2016 through August 2017, originating from 34 farms. More than 90% of the produce types collected consisted of Chinese cabbage, romaine lettuce, cucumber, and strawberry. Because of sampling limitations, smaller numbers of pepper and tomato were collected. TAB ranged from 2.77 to 5.99 log₁₀ CFU/g in fresh produce, from 6.28 to 7.81 log₁₀ CFU/g in soil, and from 1.31 to 2.98 log₁₀ CFU/g in irrigation water samples. On average, TAB for each produce type were 5.10, 5.31, 4.71, 4.89, 5.18, 4.46, and 2.77 log₁₀ CFU/g for outer leaves of cabbage, inner leaves of cabbage, romaine lettuce, cucumber, pepper, tomato, and strawberry, respectively. The differences in the TAB were attributable to produce type, especially strawberries significantly tended to have lower TAB than the other produce ($p < 0.05$) (Fig. 1). It is assumed that strawberry production is less prone to microbial contamination because greenhouses are protected from the outside environment (Holvoet et al., 2015). For irrigation water, mean TAB were present in relative low levels at 2.74, 2.98, 1.31, 2.48, 1.81, and 1.84 log₁₀ CFU/g in Chinese cabbage, romaine lettuce, cucumber, pepper, tomato, and strawberry farms, respectively (Table 2). Levels of TAB on irrigation water sample of Chinese cabbage and romaine lettuce were significantly higher than irrigation water sample of cucumber and strawberry ($p < 0.05$) (Fig. 1). Several investigators

Table 1 Culture media^a and identification methods for pathogenic bacteria isolated from fresh produce in this study

Strains	Enrichment broth	Selective agar	Identification method		
			API kit	Latex agglutination test ^b	16s rRNA sequencing ^c
<i>Bacillus cereus</i>	0.2% Bacto Peptone (Difco Laboratories, Detroit, MI, USA)	Mannitol egg yolk polymyxin agar (MYP, Difco)	API 50CHB (BioMeriux, Marcy l'Etoile, France)	— ^d	(SolGent co., Ltd., Korea)
<i>Listeria monocytogenes</i>	<i>Listeria</i> enrichment broth (Difco)	Oxford agar base (Difco) in <i>Listeria</i> selective supplement (OAB, Oxoid)	—	<i>Listeria</i> Diagnostic Reagents test kit (Oxoid, Hampshire, UK)	—
<i>Salmonella</i> spp.	Rappaport–Vassiliadis R10 broth (Difco) Tetrathionate broth (Difco)	Xylose lysine desoxycholate agar (XLD, Difco)	API 20E (BioMeriux)	—	—
<i>Staphylococcus aureus</i>	Tryptic soy broth (Difco) in 10% NaCl	Baird-Parker agar (BPA, Difco)	API Staphy (BioMeriux)	—	(SolGent co., Ltd., Korea)
<i>Escherichia coli</i> O157:H7	E.C. broth (Oxoid, Hampshire, UK)	Sorbitol MacConkey agar (SMAC, Difco)	—	<i>E. coli</i> O157 Diagnostic Reagents test kit (Oxoid)	—
<i>Clostridium perfringens</i>	Cooked meat (Difco)	Perfringens agar base (TSC, Difco)	—	—	—

^aCulture media for pathogens quoted from the MFDS Food Code

^bThe only three samples showing positive results on culture media methods for *E. coli* O157:H7 or *L. monocytogenes* were identified using Latex agglutination test method

^cSamples showing positive results with culture media methods were subsequently subjected to the 16s rRNA method

^dNot tested

have reported similar levels of TAB on fresh produce collected from farms (Johnston et al., 2005; Maffei et al., 2013). Agricultural grounds exhibited a high quantity of TAB, ranging from 6.28 to 7.81 log₁₀ CFU/g. TAB of soil samples were very similar among different farms of produce except for soil sample of strawberry (Table 2). Levels of TAB on soil sample of strawberry was significantly higher than the other soil samples ($p < 0.05$) (Fig. 1). For example, Maffei et al. (2013) found that vegetables represented TAB in levels of 5.00–7.00 log₁₀ CFU/g after harvest. Microbial loads were shown to be significantly different on fresh fruits and vegetables, depending on the type of fresh produce and the growing condition. Mostly, TAB counts are useful for indicating the shelf life duration and microbial quality of food (Mansur and Oh, 2015). Ragaert et al. (2007) reported that microbial counts at levels of > 7.00 log₁₀ CFU/g are involved in deteriorating organoleptic changes of minimally processed products, thereby resulting in consumer rejection. Microbial contamination of fresh produce can occur in any agricultural field which is fertilized with improperly processed manure (Barak and Liang, 2008). Birkhofer et al. (2008) also reported that soil is more easily accessible to microorganisms than fresh produce or irrigation water. While,

coliforms and Y/M were present in smaller numbers than TAB, the results varied greatly. The ranges for coliform or Y/M in fresh produce, soil, and irrigation water were 0–2.48, 0–3.35, and 0–0.85 log₁₀ CFU/g or 2.95–5.17, 4.51–5.27, and 0–1.15 log₁₀ CFU/g, respectively (Table 2). No differences in the coliform or Y/M were attributable to fresh produce type, although strawberries significantly tended to have fewer coliforms than the other produce ($p < 0.05$) (Fig. 1). Coliform counts varied widely among the samples within a range of 0–3.35 log₁₀ CFU/g. Some samples, such as soil or irrigation water sample of pepper, soil samples of tomato, and strawberry samples, contained no detectable coliforms at a lower limit of detection at 10 CFU/g. Steele and Odumeru (2004) reported that coliforms are widely distributed in nature and commonly found in fresh produce. Furthermore, observed numbers of yeasts and molds were lower than TAB. Oliveira et al. (2010) obtained similar results with samples of fresh produce, with yeasts and molds present in smaller counts than TAB. Yeasts and molds are associated with food spoilage; high levels may also be a health hazard because of the mycotoxins produced, and others are known to cause allergies by molds (Tournas, 2005). Our study also evaluated the presence of *E. coli* on fresh fruits and vegetables.

Table 2 Averages and ranges ($\log_{10}$ CFU/g)^a of bacterial count isolated from six different fresh produce in Korea

Quantitative ($\log_{10}$ CFU/g)							
Agricultural product	Season	Type of samples (number of samples)	TAB	Coliform	<i>E. coli</i>	Y/M	<i>B. cereus</i>
Chinese cabbage	Aug.–Oct.	Outer leaves (18)					
		Average	5.10 ± 0.69	1.96 ± 1.10	0.11 ± 0.29	4.34 ± 0.62	ND ^b
		Range	3.94–6.42	0–4.85	0–1.12	3.22–4.70	ND
		Inner leaves (18)					
		Average	5.31 ± 0.80	1.49 ± 1.20	ND	2.95 ± 1.11	ND
		Range	4.18–7.05	0–4.74	ND	0.94–4.97	ND
		Soil (16)					
		Average	6.64 ± 0.42	3.12 ± 0.93	ND	4.51 ± 0.31	0.25 ± 0.73
		Range	6.10–7.21	1.24–4.65	ND	3.65–5.07	0–2.35
		Irrigation water (16)					
		Average	2.74 ± 1.02	0.85 ± 0.77	0.35 ± 0.62	1.15 ± 1.04	– ^c
		Range	1.60–4.72	0–2.00	0–2.05	0–2.70	–
Romaine lettuce	Aug.–Oct.	Produce (20)					
		Average	4.71 ± 1.40	1.51 ± 1.81	ND	4.15 ± 0.60	0.05 ± 0.20
		Range	2.36–7.48	0–5.65	ND	3.40–6.04	0–0.91
		Soil (18)					
		Average	6.81 ± 0.29	2.56 ± 0.77	0.11 ± 0.46	4.70 ± 0.47	0.14 ± 0.57
		Range	6.43–7.48	1.30–4.39	0–1.96	4.00–5.60	0–2.43
		Irrigation water (6)					
		Average	2.98 ± 1.76	0.16 ± 0.39	ND	0.46 ± 0.61	–
Cucumber	Jul.–Sep.	Produce (14)					
		Average	4.89 ± 0.99	1.38 ± 1.01	0.02 ± 0.08	4.08 ± 1.04	0.02 ± 0.08
		Range	2.98–7.59	0–4.18	0–2.00	2.12–6.75	0–0.30
		Soil (12)					
		Average	6.84 ± 0.42	2.19 ± 1.27	0.12 ± 0.30	4.82 ± 0.81	1.12 ± 2.04
		Range	5.87–7.33	0–4.81	0–0.95	2.74–5.63	0–4.89
		Irrigation water (15)					
		Average	1.31 ± 1.12	0.36 ± 0.61	0.07 ± 0.19	0.44 ± 0.61	–
Pepper	Jul.–Aug.	Produce (2)					
		Average	5.18 ± 0.23	1.39 ± 0.35	ND	4.14 ± 0.12	ND
		Range	5.02–5.35	1.14–1.63	ND	4.06–4.23	ND
		Soil (1)					
		Average	6.28	ND	ND	4.56	ND
		Range	6.28	ND	ND	4.56	ND
		Irrigation water (1)					
		Average	2.48	ND	ND	ND	–
Tomato	Aug.	Produce (3)					
		Average	4.46 ± 0.75	2.48 ± 0.74	1.04 ± 0.73	4.32 ± 1.26	ND
		Range	3.60–4.88	1.63–2.97	0.30–1.75	3.48–5.77	ND
		Soil (2)					
		Average	6.87 ± 0.39	3.35 ± 1.94	ND	4.97 ± 0.68	2.22 ± 3.13
		Range	6.60–7.15	1.98–4.72	ND	4.49–5.45	0–4.43
		Irrigation water (2)					

Table 2 continued

Quantitative (log ₁₀ CFU/g)							
Agricultural product	Season	Type of samples (number of samples)	TAB	Coliform	<i>E. coli</i>	Y/M	<i>B. cereus</i>
Strawberry	Jan.–Mar.	Average	1.81 ± 0.29	ND	1.69 ± 0.44	1.35 ± 0.92	–
		Range	1.60–2.02	ND	1.38–2.00	0.70–2.00	–
		Produce (17)					
		Average	2.77 ± 1.22	ND	ND	4.28 ± 0.87	ND
		Range	0.70–4.28	ND	ND	0.40–5.20	ND
		Soil (17)					
		Average	7.81 ± 0.62	3.23 ± 1.34	0.12 ± 0.41	5.27 ± 0.92	0.08 ± 0.33
		Range	6.68–8.61	0.65–4.65	0–1.65	2.74–6.39	0–1.35
		Irrigation water (15)					
		Average	1.84 ± 0.59	0.17 ± 0.31	0.02 ± 0.08	0.59 ± 0.56	–
		Range	0.95–3.30	0–1.09	0–0.30	0–1.73	–

^aData represent mean ± standard deviations of two measurements

^bNot detected

^cNot tested

On fresh produce and in irrigation water, populations of *E. coli* were almost below the detection limits (< 10 CFU/g) (Table 2; Fig. 1). Abadias et al. (2007) found similar results, with *E. coli* present in 7.1% of whole vegetables and 11.4% of fresh-cut samples. Additionally, *E. coli* was isolated from 8.9% of the samples of Norwegian organically grown lettuce (Loncarevic et al., 2005). Other studies assessing the bacteriological quality of fresh produce also found that the number of *E. coli* present was usually low (Johannessen et al., 2004; Mukherjee et al., 2004). However, the highest *E. coli* counts were observed in the soil samples of romaine lettuce, cucumber, and strawberry (Table 2; Fig. 1). Other studies also reported that high levels of *E. coli* and coliform were found in many types of soils, sediments, and plants (Selma et al., 2007; Semenov et al., 2009). In addition, the presence of visible soil particles between the leaves in lettuces, leafy greens, and cabbages might have resulted in significantly higher *E. coli* prevalence in these produce (Semenov et al., 2009). The contamination of produce can occur in the field by contaminated soil and deposition of feces by wild animals (Holvoet et al., 2014). *E. coli* was analyzed as a potential indicator of fecal contamination and risk of emerging pathogens, such as *E. coli* O157:H7 (Selma et al., 2007). The high incidence of *E. coli* in samples was reason for concern, exposing the population to contamination (Maffei et al., 2013; Stenfors Arnesen et al., 2008).

The prevalence of pathogens in fresh produce, soil, and irrigation water samples

In this study, *B. cereus* or *S. aureus* were detected in samples of fresh produce, soil, and irrigation water

(Table 3), which is most frequently linked to produce-related foodborne disease outbreaks (Bennett et al., 2013). However, *L. monocytogenes*, *Salmonella* spp., *E. coli* O157:H7, and *C. perfringens* were not detected in any of the sources, including fresh produce, soil, and irrigation water samples. This result is consistent with data reported in the U.S. Food and Drug Administration's (FDA) microbiological sampling assignment (FDA, 2017), showing that less than 1.0% sprouts were contaminated with pathogenic bacteria, such as *E. coli* O157:H7 and *S. enterica* serovar Enteritidis. Table 3 showed that the *B. cereus* was isolated from produce, soil, and irrigation water samples of Chinese cabbage, romaine lettuce, cucumber, tomato, and strawberry in 15 (7.2%) out of 195 samples. *B. cereus* is commonly present in food production environments by virtue of its highly adhesive endospores, spreading to all kinds of food (Stenfors Arnesen et al., 2008). In particular, *B. cereus* was more prevalent in the soil samples of Chinese cabbage and cucumber farms, compared to the produce or irrigation water samples (Table 3). While, on fresh produce and in irrigation water, populations of *B. cereus* were almost below the detection limits (< 10 CFU/g) (Table 2; Fig. 1). In addition, *S. aureus* was detected in 3.6% of the total samples, and the most contaminated samples were the irrigation water samples of cucumber farms. Among fresh produce samples, cucumber samples (product, soil, irrigation water) had slightly higher counts of *B. cereus* or *S. aureus* (Table 3). *S. aureus* and *B. cereus* have been detected on raw vegetables and are known to be carried by food handlers or contaminated soil environment, respectively (Bae et al., 2011; Bartoszewicz et al., 2008; Beuchat, 2002).

Fig. 1 Numbers ($\log_{10}$ CFU/g) of contaminating microbes in (A) Chinese cabbage, (B) Romaine lettuce, (C) cucumber, and (D) strawberry. Prevalence was compared between sample types; sample types with different letters were significantly different

($p < 0.05$). Bars represent the standard deviation. There was significant difference on levels of total aerobic bacteria and coliform between Chinese cabbage, Romaine lettuce, cucumber, and strawberry ($p < 0.05$)

Related to the detection method, all samples were analyzed for six different pathogens by various isolation methods including the selective agar method, API kit, Latex agglutination test, and 16s rRNA sequencing method (Table 4). Of the total 195 samples of fresh produce, soil, and irrigation water investigated, 114 samples were detected on selective agar. Among positive samples, 40, 30, 26, 16, and 2 samples were detected for *B. cereus*, *L. monocytogenes*, *S. aureus*, *E. coli* O157:H7, and *C. perfringens* on selective agar, respectively. However, their phenotypic properties greatly differed from those of typical samples on each selective agar. Positive samples on selective agar were further identified as false positives based on the biochemical identification assay using the API kit. In addition, positive colonies that developed on MYP

or BPA were confirmed as *B. cereus* or *S. aureus* as a consequence of 16s rRNA sequencing, respectively. However, other positive colonies developed on OAB, XLD, SMAC, and TSC were determined as results of false positives using 16s rRNA sequencing method. The 16s rRNA sequencing method detected more negative results than the selective agar method and Latex agglutination test (Table 4). This resulted in a higher specificity for the 16s rRNA sequencing method with a false-positive rate. According to a study conducted by Bae et al. (2011), where the raw vegetable samples were analyzed by selective agar method, a higher number of positive results were obtained with the selective agar method compared with that obtained by the PCR. These identification methods will be useful in screening of various fresh produce for foodborne

Table 3 Prevalence of pathogenic contamination in fresh produce

Agricultural product	Season	Type of samples (number of samples)	Qualitative [N (%)]					
			<i>B. cereus</i>	<i>L. monocytogenes</i>	<i>Salmonella</i> spp.	<i>S. aureus</i>	<i>E. coli</i> O157:H7	<i>C. perfringenes</i>
Chinese cabbage	Aug.–Oct.	Produce (18)	ND ^a	ND	ND	1 (5.6%)	ND	ND
		Soil (16)	3 (16.7%)	ND	ND	1 (5.6%)	ND	ND
		Irrigation water (16)	ND	ND	ND	1 (6.3%)	ND	ND
Romaine lettuce	Aug.–Oct.	Produce (20)	1 (5.0%)	ND	ND	1 (5.0%)	ND	ND
		Soil (18)	1 (5.6%)	ND	ND	ND	ND	ND
		Irrigation water (6)	ND	ND	ND	ND	ND	ND
Cucumber	Jul.–Sep.	Produce (14)	1 (7.1%)	ND	ND	ND	ND	ND
		Soil (12)	6 (50.0%)	ND	ND	ND	ND	ND
		Irrigation water (15)	ND	ND	ND	3 (20.0%)	ND	ND
Pepper	Jul.–Aug.	Produce (2)	ND	ND	ND	ND	ND	ND
		Soil (1)	ND	ND	ND	ND	ND	ND
		Irrigation water (1)	ND	ND	ND	ND	ND	ND
Tomato	Aug.	Produce (3)	ND	ND	ND	ND	ND	ND
		Soil (2)	ND	ND	ND	ND	ND	ND
		Irrigation water (2)	1 (50.0%)	ND	ND	ND	ND	ND
Strawberry	Jan.–Mar.	Produce (17)	1 (5.9%)	ND	ND	ND	ND	ND
		Soil (17)	1 (5.9%)	ND	ND	ND	ND	ND
		Irrigation water (15)	ND	ND	ND	ND	ND	ND
Total of qualitative [N (%)] (195)			14 (7.2%)	0 (0.0%)	0 (0.0%)	7 (3.6%)	0 (0.0%)	0 (0.0%)

^aNot detected**Table 4** Numbers of positive samples with pathogenic contamination of fresh produce

Agricultural product	Type of samples (number of samples)	Numbers of positive samples											
		<i>B. cereus</i>		<i>L. monocytogenes</i>		<i>Salmonella</i> spp.		<i>S. aureus</i>		<i>E. coli</i> O157:H7		<i>C. perfringens</i>	
		SA ^a (API)	16s rRNA ^b	SA (Latex test) ^c	16s rRNA	SA (API)	16s rRNA	SA (API)	16s rRNA	SA (Latex test)	16s rRNA	SA (API)	16s rRNA
Chinese cabbage	Produce (18)	2 (–) ^d	0 ^e	0	–	0	–	2 (–)	1	0	–	0	–
	Soil (16)	7 (–)	3	0	–	0	–	3 (–)	1	1 (–)	0	0	–
	Irrigation water (16)	0	–	2 (0)	0	0	–	1 (–)	1	1 (–)	0	1 (–)	0
Romaine lettuce	Produce (20)	2 (–)	1	2 (1)	0	0	–	5 (–)	1	1 (–)	0	0	–
	Soil (18)	3 (–)	1	1 (0)	0	0	–	0 (–)	–	1 (–)	0	0	–
	Irrigation water (6)	2 (–)	0	1 (0)	0	0	–	0 (–)	–	1 (–)	0	0	–
Cucumber	Produce (14)	3 (–)	1	8 (0)	0	0	–	9 (1)	0	2 (–)	0	0	–
	Soil (12)	9 (–)	6	9 (0)	0	0	–	2 (–)	0	5 (–)	0	0	–
	Irrigation water (15)	0	–	7 (2)	0	0	–	3 (–)	3	4 (–)	0	1 (–)	0
Pepper	Produce (2)	0	–	0	–	0	–	1 (–)	0	0	–	0	–
	Soil (1)	1 (–)	0	0	–	0	–	0	–	0	–	0	–
	Irrigation water (1)	0	–	0	–	0	–	0	–	0	–	0	–

Table 4 continued

Agricultural product	Type of samples (number of samples)	Numbers of positive samples											
		<i>B. cereus</i>		<i>L. monocytogenes</i>		<i>Salmonella</i> spp.		<i>S. aureus</i>		<i>E. coli</i> O157:H7		<i>C. perfringens</i>	
		SA ^a (API)	16s rRNA ^b	SA (Latex test) ^c	16s rRNA	SA (API)	16s rRNA	SA (API)	16s rRNA	SA (Latex test)	16s rRNA	SA (API)	16s rRNA
Tomato	Produce (3)	1 (–)	0	0	–	0	–	0	–	0	–	0	–
	Soil (2)	1 (–)	1	0	–	0	–	0	–	0	–	0	–
	Irrigation water (2)	0	–	0	–	0	–	0	–	0	–	0	–
Strawberry	Produce (17)	5 (–)	0	0	–	0	–	0	–	0	–	0	–
	Soil (17)	4 (–)	1	0	–	0	–	0	–	0	–	0	–
	Irrigation water (15)	0	–	0	–	0	–	0	–	0	–	0	–

^aSA, isolation methods using selective agar; API, identification methods using API kit. Numbers of positive samples using the selective agar method (numbers of positive samples in API kit method among positive samples in selective agar method)

^bIdentification methods using 16s rRNA sequencing method. Numbers of positive samples in 16s rRNA sequencing method among positive samples in selective agar method

^cIdentification methods using Latex test kit. Numbers of positive samples in selective agar method

^dNot tested

^eNot detected

pathogens, and the use of uniform enrichment protocols will allow for a continuation of culture methods for confirmation of presumptive positive samples.

In conclusion, the present study revealed that the field soil or irrigation water around farms did not have direct contact with the contaminated product. For the microbiological quality, higher levels of TAB and Y/M were detected on fresh produce, soil, and irrigation water samples. However, the variability in the coliform counts was not related to an increase in TAB nor was it indicative of the presence of pathogens. Furthermore, *B. cereus* and *S. aureus* showed the highest levels in irrigation water and soil samples. This indicates that while the use of fertilizer and compost provides nutrients for the growth of fresh produce, it also may be the cause of *B. cereus* contamination. Fresh produces are exposed to a variety of different conditions during growth, harvest, preparation, and distribution that could increase natural contamination. With the urgent (or gradual) advent of climate change, such as global warming in East Asian countries, foodborne disease outbreaks involving fresh produce are increasing in severity, particularly over the past 5 years in Korea. Thus, the importance of conducting regularly programmed microbial risk assessment of fresh fruits and vegetables is magnified. Results obtained from the present study will provide information that can be used to better understand the microbiological behavior and ecology in agricultural farming fields.

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