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Article

Monitoring of Bacterial Pathogens in Agricultural Products and Environments at Farms in Korea

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A total of 142 samples comprising vegetables, soil, and water collected from different agricultural farms (five provinces) were analyzed for total aerobic bacteria (aerobic plate count [APC]), *Bacillus cereus*, *Escherichia coli*, *Listeria monocytogenes*, *Salmonella* spp., and *Staphylococcus aureus*. The average of total APC in all the samples ranged from 4.72×10^5 to 8.62×10^8 CFU/g (mL). The prevalence of *B. cereus*, *E. coli*, *L. monocytogenes*, *S.* spp., and *S. aureus* for all samples was 17.60%, 2.11%, 1.4%, 0%, and 2.11% respectively, and their counts averaged to 4.87×10^4 CFU/g (mL), 4.34×10^3 CFU/g (mL), 2.15×10^2 CFU/g, 0 CFU/g, and 3.12×10^3 CFU/g respectively. Among the 3 different types of samples, 6 vegetables (10.34%), 24 soil (38.70%), and 3 water (13.64%) samples were found to be positive for bacterial pathogens. The result showed that the occurrence of bacterial pathogen in the samples analyzed was low. Further time to time monitoring and need to wash of raw agricultural products is recommended.

Key words: *aerobic plate count, bacterial pathogens, soil, water, monitoring, vegetables*

Agricultural products such as fruits and vegetables are in great demand in Korea, and are included in everyday servings. According to Food and Agriculture Organization (FAO), fruits and vegetables consumption and production in Korea has been increasing [FAO, 2007]. Besides increase in consumption, issue of public health concern has arisen. Accordingly, number of foodborne illness outbreaks caused by consumption of bacterial contaminated fruits and vegetables has increased. The Korea Food and Drug Administration (KFDA) reported that number of food poisoning outbreaks associated with fruits and vegetables increased from 1.23% to 5.77% and number of patients increased from 1.43% to 10.66%, from 1996 to 2000 [Park *et al.*, 2001].

A broad variety of agricultural products has been linked to various pathogens. The most common outbreaks have been caused by pathogens, namely *Bacillus cereus*, pathogenic *Escherichia coli*, *Listeria monocytogenes*, *Salmonella* spp., and *Staphylococcus aureus* [Beuchat, 1996; Fang *et al.*, 1999; Heisick *et al.*, 1989].

Pathogens may contaminate agricultural products at all stages from farm to table; during production, harvest, processing, and transportation, as well as in retail and food service establishments [FDA, 2004; Ruiz *et al.*, 1987]. However, there is a recognized potential for the on-farm transfer of pathogens to agricultural products during primary production [Beuchat, 1996]. Soil, faecal matter of both domestic as well as wild animals, contaminated water used for irrigation, floodwater, and surface water has been identified as a source of contamination [Beuchat, 1996; FDA, 2004]. In addition, the use of untreated manure or sewage, lack of field sanitation, and contamination by handlers are also suggested as potential contributing factors [FDA, 2004].

Most of the studies on microbial quality of agricultural products in Korea are focused on retail and post-harvest samples [Kim *et al.*, 2005a; Kim *et al.*, 2004; Lee *et al.*, 2005]. There are limited studies on agricultural products at the farm. The microbial analysis of the agricultural

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products on the farm including the agricultural environment would enhance our understanding of bacterial condition and factors that contribute to contamination. Considering that bacterial pathogens present in agricultural environment can transfer to agricultural products and pose a health hazard to consumers, the objective of this study was to provide an initial estimation on microbiological condition of agricultural products including environments (soil and water) based on collection of samples directly from different agricultural farms.

Materials and Methods

Sample collection. A total of 142 samples, comprising vegetables (58), soil (62), and water (22) were collected at random from different agricultural farms (from five provinces) during harvest seasons between July and October, 2005 (Table 1). Because of sampling limitations, smaller numbers of some samples were collected. All the samples were collected directly from the farms [both upland (pepper) and paddy (rice)] and kept in plastic bags. Vegetable samples were transferred without being washed or soil particles rubbed off. The equipments and instruments used during sampling were sterilized to prevent cross contamination. All samples collected were shipped on ice to laboratory and microbiological analysis was initiated within 48 h of sample collection.

Microbiological analysis. Twenty five grams (mL) of samples were aseptically transferred to 225 mL of buffered peptone water (BPW; Merck KGaA, Germany) and Listeria enrichment broth (LEB; Merck). BPW was used as primary enrichment for *B. cereus, E. coli, Salmonella* spp., and *S. aureus* and LEB used for *L. monocytogenes.* Vegetable samples were blended in sterile blender for 2 minutes and transferred to Whirl-Pack bags (Nasco, Fort Atkinson, WI), while soil and water samples were kept in Whirl-Pack bags and swirled for 2-5 minutes. Enumeration of total aerobic bacteria (aerobic plate count [APC]) was carried out by surface plating 100 μL of dilutions (serially diluted) on plate count agar (PCA; Merck), plates incubated at 30°C for 24 h.

Enumeration and identification of *B. cereus* was done by surface plating 100 μL of inoculum on mannitol egg yolk polymyxin agar (MYPA; Merck) plates (50,000 IU of polymyxin per liter). After 24 h of incubation at 30°C, the plates were examined for typical pink colonies with irregular edge surrounded by white area. These colonies were analyzed for β-hemolysis on blood agar (BA; Merck) with 5% sheep blood.

Coli ID (Merck) and Sorbitol Macconkey agar containing potassium tellurite and cefixime (SMA-TC; Merck) were used for enumeration and identification of *E. coli* and *E. coli* O157:H7, respectively. 1000 μL (one mL) of diluted inoculum was spreaded in each petri plates and approximately 15 mL of molten coli medium added (maintained at approx. 47°C), mixed carefully and allowed to dry and 100 μL of diluted inoculum was surface plated in SMA-TC agar plates. Coli ID plates were incubated at 44°C and SMA-TC at 37°C for 24 h. Pink colonies on Coli ID and sorbitol-negative colonies on SMA-TC were assumed to be positive for *E. coli* and serotype O157:H7 respectively.

L. monocytogenes was enumerated and identified using two successive steps. 100 μL of enrichment broth was inoculated for secondary enrichment in Fraser broth (Merck) and incubated at 37°C for 24 or 48 h. Positive Fraser broth with black coloration were streaked on Oxford agar (Merck) and were examined for typical colonies (black, shiny, convex with narrow zone of opacity) after 48 h of incubation at 37°C and were also subjected to CAMP (Christie, Atkins, Munch, Petersen) test. For *Salmonella* spp., 100 μL of enrichment broth was inoculated in Rappaport-Vassiliadis (RV; Merck) broth as a sec-enrichment and incubated at 42°C for 24 h. Positive cultures were serially diluted, 100 μL inoculum was spread on Hektoen enteric agar (HEA; Merck) and Rambach agar (Merck) and the plates were incubated at 37°C for 24 h. Blue-green to blue colonies with or without black centers on HEA and red colonies on Rambach agar were assumed as presumptive *Salmonella* spp. isolates.

Enumeration and identification of *S. aureus* was done by surface plating 100 μL of inoculum (after serial dilution) on Baird parker agar (BPA; Merck) and plates were incubated at 37°C for 48 h. The plates were examined for typical *S. aureus* colonies (black, shiny, convex with narrow zone of opacity surrounded by zone of clearing). These colonies were further cultured on mannitol salt agar (MSA; Merck) and blood agar (Merck) for mannitol utilization and hemolytic character and also tested for coagulase-positive staphylococci. Positive controls obtained from Korean Culture Center of Microorganisms (KCCM), Seoul, Korea were simultaneously processed to assist in identification process. Typical bacterial colonies obtained from different selective agar were biochemically characterized using API kits.

Polymerase chain reaction (PCR) was applied to determine the virulence genes of pathogenic bacterial isolates. Total genomic DNA of bacteria monitored was isolated using modified protease-sodium dodecyl sulfate (SDS) lysis procedure [Sambrook and Russell, 2001]. The primers shown in Table 2 were used to amplify pathogenic genes [Guinebretiere *et al.*, 2002; Oswald *et al.*, 2000; Steven *et al.*, 2002; Malorny *et al.*, 2004;

Berger-Bachi *et al.*, 1989]. Amplification was carried out in 25 μL reaction volumes, containing 20 *p*mol of each primer, 20 μM concentration of each dNTPs (dATP, dCTP, dGTP, and dTTP) (Promega Co.), 5 units of Taq polymerase (Biotools Co.) with 10 ng of plasmid DNA. PCR analysis was performed with DNA thermal cycler (Perkin-Elmer Applied Biosystems, foster City, CA, USA). Amplification conditions were followed as listed in Table 3. A 5 μL of each amplified PCR products were electrophoresed on a 0.7% agarose gel (Qbiogene, Irvine, CA, USA) stained with 0.5 μg/L ethidium bromide and visualized on a UV transilluminator. The resultant PCR products were excised from the gel and purified using a Geneclean® Turbo kit (Qbiogene, Irvine, CA, USA). Purified DNAs were ligated into the pGEM-T easy vector (Promega Co. Madison, WI, USA). Plasmids containing the DNA regions were then send for sequencing. DNA homology searches were carried out with the NCBI databases, using the BLAST network service [Altschul *et al.*, 1990].

Results

Sample collection. A total of 142 vegetables and environmental samples were collected at random from different agricultural farms (from five provinces) during harvest seasons between July and October, 2005. Samples consisted different types of vegetables, soil (both upland and paddy), and water (both upland and paddy). Because of sampling limitation small number of some samples was collected. Table 1 specifies number of sample stratified by sample origin.

Microbiological analysis. Total APC in all the samples

Table 1. Sampling sites and various types of samples collected at farms from five provinces

Samples	Provinces				
	Gangwan-province (July)	Jeolla-province (August)	Chungcheong-province (August)	Gyeongsang-proovince (September)	Gyeonggi-province (October)
Vegetable	Pepper (6) Potato (4) Carrot1 (2) Radish (1)	Pepper (4) Carrot (3)	Pepper (5) Potato (4)	Pepper (6) Potato (5)	Pepper (8) Potato (6) Carrot (2) Radish (2)
Soil	Pepper (6) Rice(5) Potato (2) Carrot (2) Radish (1) Bean (1)	Pepper (5) Rice (5)	Pepper (5) Rice (6)	Pepper (6) Rice (5) Potato (1) Bean (1)	Pepper (4) Rice (4) Potato (1) Radish (1) TPS[a] (1)
Water	Rice (3)	Rice (4)	Pepper (2) Rice (4)	Rice (5)	Rice (4)

[a]TPS-Thermal Power Station

Table 2. Nucleotide sequence and anticipated size of PCR for selective pathogens

Bacteria	Primer target and direction	Oligonucleotide sequence (5'-3')	Size of Amplified products (bp)	References
B. cereus	HAF	AAGCAATGGAATACAATGGG	2682	12
	HBR	AATATGTCCCAGTACACCCG		
E. coli	SK1,F	CCCGAATTCGGCACAAGCATAAGC	832	13
	SK2, R	CCCGGATCGGTCTCGCCAGTATTCG		
L. monocytogenes	inlAF1, F	CCGCCTAATGGGAAAGTAAA	2235	14
	inlAR1, R	AGGCGGAGATGCTGGTG		
Salmonella spp.	Malo2-F	TATTGTTGATTAATGAGATCCG	373	15
	Malo2-R	ATATTACGCACGGAAACACGTT		
S. aureus	GFEMAR-1, F	AAAAAAGCACATACCAAGCG	132	16
	GFEMAR-2,R	GATAAAGAAACCAGCAG		

F-forward, R-reverse

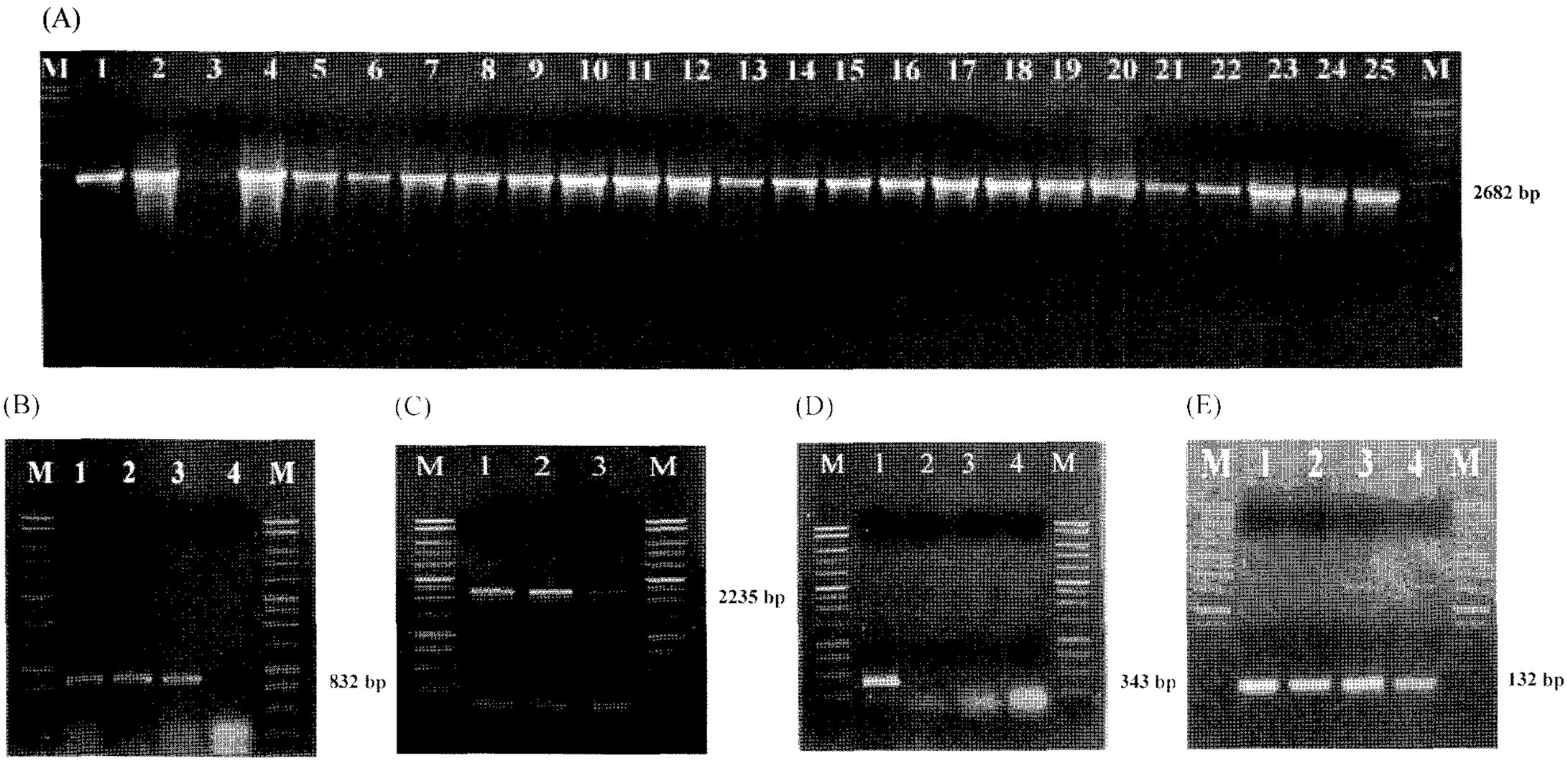

Fig. 1. Agarose gel electrophoresis of the PCR amplification products of bacterial isolates from vegetable, soil, and water samples. (A) *B. cereus*: Lane M, Marker; Lane 1, *B. cereus* KCCM 1174; Lane 2, Pepper soil; Lane 3, Pepper; Lane 4, Rice soil; Lane 5, Pepper soil; Lane 6, Rice soil; Lane 7, Rice soil; Lane 8, Rice soil; Lane 9, Rice soil; Lane 10, Pepper soil; Lane 11, Pepper soil; Lane 12, Rice soil; Lane 13, Rice water; Lane 14, Rice water; Lane 15, Radish; Lane 16, TPS soil; Lane 17, Carrot; Lane 18, Rice soil; 19, Pepper soil; Lane 20, Pepper; Lane 21, Rice soil; Lane 22, Pepper soil; Lane 23, Rice soil; Lane 24, Rice soil; Lane 25, Rice soil; Lane 26, Rice soil. (B) *E. coli*: Lane M marker; Lane 1-3; PCR for Lane 1, Rice water; Lane 2- Rice soil; Lane 3, Thermal Power generation soil; Lane 4, strain used in this study (non pathogenic strain obtained from KCCM (KCCM 12119). (C) *L. monocytogenes*: Lane M, marker; Lane 1-3; Lane 1, *L. monocytogenes* KCCM 40307; Lane 2, Potato; Lane 3, Rice soil. (D) *Salmonella* spp.: Lane M-marker; Lane 1, *S. cholerasesuis* KCCM 11806; Lane 2, 3, 4, negative results. (E) *S. aureus*: Lane M- marker; Lane 1, *S. aureus* KCCM 12103; Lane 2, Pepper, Lane 3, Pepper, Lane 4, Pepper soil.

ranged from geometric mean of 4.72×10^5 to 8.62×10^8 CFU/g (mL) (Table 4). Total APC was higher in soil samples than those in vegetable and water samples. Among the vegetable samples, total APC level was higher on potato than those on pepper. The total APC level for potato was higher in those collected from Gangwon-province (July) and Gyeonggi-province (October) than from other provinces, while for pepper it was higher in those collected from Gangwon-province (July) than from other provinces. Among the soil samples, paddy soil had the higher APC level than upland soil, which increased in July (Gangwon-province), decreased in September (Gyeongsang-province) and again increased in October (Gyeonggi-province). Similar level of APC was found in the water samples collected from the farm, showing similar trend as in soil samples with higher bacterial count in paddy water (rice water) during July and October, and lower during august.

Pathogens detected in the present study were *B. cereus*, *E. coli*, *L. monocytogenes,* and *S. aureus* (Table 5). Biochemical characterization of these bacterial pathogens was done using API kits (data not shown) and the

Table 3. PCR conditions

Bacteria	Gene[a]	Initial denaturation	Amplification (no. of cycles)	Final hold
B. cereus	Hbl	94°C, 2 min	94°C, 30 sec; 58°C, 30 sec; 68°C, 1 min (30)	68°C 7 min
E. coli	eae	95°C, 4 min	95°C, 3 min; 66°C, 1 min; 72°C, 2.5 min, (35)	72°C 7 min
L. monocytogenes	inlA	94°C, 2.30 min	94°C, 30 sec; 54°C, 30 sec; 72°C, 1 min (40)	72°C 7 min
Salmonella spp.	InvA	95°C, 3 min	95°C, 1 min; 59°C, 1 min; 72°C, 30 sec (30)	72°C 5 min
S. aureus	femA	94°C, 5 min	94°C, 2 min; 57°C, 2 min; 72°C, 1 min (35)	72°C 7 min

[a]gene encoding pathogenicity for bacterial pathogens, *Hbl*-haemolysin gene, *eae*-intiment gene, *inlA*-virulence gene, *InvA*-invasive gene, *femA*-methicillin resistant gene

Table 4. Total aerobic plate counts in vegetable, soil and water samples

Samples	Total plate count [Nb CFU/g [c] (×10^6)]				
	Gangwon-province (July)	Jeolla-province (August)	Chungcheong-province (August)	Gyeongsang-province (September)	Gyeonggi-province (October)
Vegetables					
Pepper (n[a]=29)	(6) 4.96	(4) 3.62	(5) 0.472	(6) 0.674	(8) 0.778
Potato (19)	(4) 56.25	NA[d]	(4) 6.26	(5) 6.47	(6) 75.02
Carrot (7)	(2) 5.04	(3) 6.42	NA	NA	(2) 5.64
Radish (3)	(1) 76.01	NA	NA	NA	(2) 5.23
Soil					
Pepper (26)	(6) 73.21	(5) 34.82	(5) 58.36	(6) 42.57	(4) 28.46
Rice (25)	(5) 862.63	(5) 623.30	(6) 42.66	(5) 64.08	(4) 778.36
Potato (4)	(2) 43.78	NA	NA	(1) 35.01	(1) 64.21
Carrot (2)	(2) 31.06	NA	NA	NA	NA
Radish (2)	(1) 64.82	NA	NA	NA	(1) 4.54
Bean (2)	(1) 42.46	NA	NA	(1) 21.25	NA
TPS[e] (1)	NA	NA	NA	NA	(1) 448.03
Water					
Pepper(2)	NA	NA	(2) 0.821	NA	NA
Rice (20)	(3) 671.36	(4) 483.29	(4) 53.68	(5) 36.31	(4) 324.27

[a]No of samples analyzed, [b]Positive samples, [c]CFU-Colony forming unit, [d]NA- Not analyzed, [e]TPS- Thermal Power Station

virulence genes were detected using PCR (Fig. 1). A blast search homology of pathogenic gene sequence (pathogenic gene as described in Table 3) identified the strains as *B. cereus*, *E. coli*, *L. monocytogenes,* and *S. aureus* (>98% sequence similarity). The prevalence of these pathogens for all samples was 17.60, 2.11, 1.4, and 2.11% respectively, and their count averaged to 4.87×10^4 CFU/g (mL), 4.34×10^3 CFU/g (mL), 2.15×10^2 CFU/g, and 3.12×10^3 CFU/g respectively. However, *Salmonella* spp. were not detected in any of the samples. *B. cereus* was detected in samples collected from all five provinces, while *E. coli*, *L. monocytogenes,* and *S. aureus* were detected only in samples collected from Gyeonggi-province, Gangwon-province, and Gyeongsang-province, respectively. Among the 3 different types of samples, 6 vegetables (10.34%), 24 soil (38.70%), and 3 water (13.64%) samples were found to be positive for these pathogens.

Discussion

Many pathogenic microorganisms are natural inhabitants of soil and some others inhabitants of the gastrointestinal tract of livestock, and animal manure have been identified as a vehicle for transmission of pathogens to agricultural products. The present study was conducted to generate data on microbiological condition of agricultural products and environments at farm. The total APC ranged from geometric mean of 4.72×10^5 to 8.62×10^8 CFU/g (mL) (Table 4). Soil had higher APC level than water and vegetables. The total APC in vegetables averaged to 10^7 CFU/g which in general is consistent to those of other studies that examined microbial level on fresh produce items. Several investigators have reported similar level of APC on green vegetables collected from production and retail establishment [Lynette *et al.*, 2006; PHLS, 2000]. For example, Ruiz *et al.* (1987) in their survey found APC count ranging from 10^5 to 10^7 CFU/g on field samples and from 10^4 to 10^6 CFU/g on retail samples. Among the soil samples, paddy soil had the higher APC level than upland soil which could be due to the difference in physiochemical and nutrient status of paddy soil, thus supporting the growth of microorganisms [Kim *et al.*, 2005b]. The difference in APC in different province by months, could be deduced to, flood contamination during July in Gangwon-province increasing the bacterial count, water-logging during September in Gyeongsang-province (creating anaerobic condition, limiting the growth of aerobes and diluting soil nutrients) decreasing the bacterial count and, drying of soil during October in Gyeonggi-province, favoring proliferation of bacteria (by aeration and organic matters from dead roots and plant debris) and thus increasing the number [Gaunt *et al.*, 1995]. The total APC in soil samples in our study averaged to 10^7 CFU/g, similar to those detected by Kim *et al.* (2005) (10^7 cell/g) but lower than those detected by Gaunt *et al.* (1995) where they found APC averaging to 7.6×10^{10} CFU/g in Japanese paddy soil and 9.8×10^9 CFU/g in Philippine water-logged paddy soil.

Table 5. Prevalence of pathogenic bacteria in vegetable, soil and water samples

Samples	*B. cereus*	*E. coli*	*L. monocytogenes*	*Salmonella.* spp	*S. aureus*
	N[a] CFU/g[b](×10⁴)	N CFU/g(×10³)	N CFU/g(×10²)	N CFU/g	N CFU/g(×10³)
Gangwon-province					
Vegetables(13)	(1) 0.353	ND[c]	(1) 2.20	ND	ND
Soil (17)	(5) 4.24	ND	(1) 2.1	ND	ND
Water (3)	ND	ND	ND	ND	ND
Jeolla- province					
Vegetables (7)	ND	ND	ND	ND	ND
Soil (10)	(3) 4.92	ND	ND	ND	ND
Water (4)	ND	ND	ND	ND	ND
Chungcheong-province					
Vegetables (9)	ND	ND	ND	ND	ND
Soil (11)	(4) 6.08	ND	ND	ND	ND
Water (6)	ND	ND	ND	ND	ND
Gyeongsang-province					
Vegetables (11)	ND	ND	ND	ND	(2) 2.43
Soil (13)	(5) 6.41	ND	ND	ND	(1) 3.84
Water (5)	(1)0.674	ND	ND	ND	ND
Gyeonggi-province					
Vegetables (18)	(2) 6.62	ND	ND	ND	ND
Soil (11)	(3) 5.36	(2) 3.45	ND	ND	ND
Water (4)	(1) 0.267	(1) 6.14	ND	ND	ND

[a]Positive samples, [b]CFU-Colony forming unit, [c]ND- Not detected

B. cereus was detected in 25 (17.60%) samples, and majority of them were soils. Prevalence in vegetables, soil, and water was 5.17, 32.25, and 9.09%, respectively. The incidence level averaged to 10^4 CFU/g (mL) for all the samples, consistent with those found by Watanabe and Hayano (1995) (10^5 to 10^7 CFU/g) in rice and wheat rotation soils, and Fang *et al.* (1999) (10^3 CFU/g) in different vegetarian food products. Vegetable samples positive for *B. cereus* were pepper, carrot and radish. Incidence level was higher in carrot and radish which were grown in direct contact with the soil than in pepper grown above soil. Recovery of this bacterium in the samples at farm is not surprising considering its common association with the soil.

In the present study, enteropathogenic *E. coli* was detected in 2.11% of the samples collected and all the samples were from Gyeonggi-province. It was present in soil and water samples averaging 10^3 CFU/g (mL). Its presence could be as a result of faecal contamination (animal, human or bird). Animals and birds are natural reservoir of this bacterium, and can be transmitted to soil or water through faeces, and in human by faecally contaminated food or direct contact with animal [Akten *et al.*, 2004]. *E. coli* normally is not a human pathogen, its presence indicates occurrence of faecal contamination at some point through production to consumption and is often used for monitoring the sanitary conditions of food processing. However, some strains have gained genetic changes making them pathogenic strain. Pathogenic strain of *E. coli* was not detected in vegetable samples, and serotype O157:H5 was not detected in any of the samples analyzed, which is similar to the survey on various vegetables (nearly 500 samples) by Kim *et al.* (2005a) where they did not detect any pathogenic strain of *E. coli* and serotype *E. coli* O157:H5 in any of the samples analyzed.

L. monocytogenes is ubiquitous in nature and has been isolated from a variety of environmental sources, including surface water, soil, sewage, food-processing plants and agricultural soils, and has also been frequently isolated from plant vegetation including raw vegetables [Dowe *et al.*, 1997; Fenlon, 1999]. Heisick *et al.* (1989) reported the highest frequency of *L. monocytogenes* in potato and radish. In our study, it accounted for the lowest prevalence (1.4%), and was detected in 2 out of 142 samples (only in samples collected from Gangwon-province) with incidence level averaging to 10^2 CFU/g. Its occurrence in potato tubers collected from the retail markets has been previously reported [Heisick *et al.*, 1989]. Absence of *L. monocytogenes* in some vegetables may be explained by

less contact with the soil and in some as it is in carrots could be due to the antibacterial effect of some component of these vegetables [Nguyen-the and Lund, 1992]. These hypotheses are in accordance with our present study where *L. monocytogenes* was isolated in rice soil and potato and not in pepper, radish and carrot.

Absence of *Salmonella* spp. in all the samples analyzed is promising. Though *Salmonella* is the bacteria causing the highest food poisoning illness in Korea, its absence at farm indicates good livestock management and good use of manure. Failure to detect this pathogen could be due to their absence in the soil or due to unfavorable environmental conditions for their survival or growth under the field conditions [Unc and Goss, 2004]. Lack of isolation of this pathogen in organic and conventional vegetables has been described in previous study [Mukherjee *et al.*, 2004]. Though *Salmonella* spp. were not detected in the present study, these have been isolated from variety of fresh produce mostly in low levels. In a survey conducted by FDA [FDA, 2003] with 1,028 domestically produced fruits and vegetables, *Salmonella* spp. were detected in 1 of 142 lettuce samples and 1 of 85 cilantro samples. Generally, prevalence and contamination level of *Salmonella* spp. are considered to be low in fresh vegetables and cereals than in meat and other meat products [Baird-Parker, 1990].

S. aureus is known to be carried by food handlers (up to 50%) which reside in respiratory passages, skin and nasal membranes [Jablonski and Bohach, 1997]. It contributes to contamination of agricultural products due to the errors in human handling. Isolation of this bacterium in vegetables has been reported earlier [Fang *et al.*, 1999; Johnston *et al.*, 2005]. In our study, *S. aureus* was detected in 2.11% of the samples analyzed collected from Gyeongsang-province. It was detected in pepper and pepper-growing soil with incidence levels averaging 10^3 CFU/g. Fang *et al.* (1999) in their study on different vegetarian food products found higher levels of the bacterium than we did in our study. High level of *S. aureus* is required to produce toxin and cause disease, levels higher than 10^4 CFU/g are potentially hazardous [PHLS, 2000].

In the present study, the presence of high plate count and some bacterial pathogens in vegetable samples could be due to the fact that, the samples were collected with the soil debris in it and were not subjected to any treatment. Washing would have lowered the incidence of the bacterial population. Climatic differences of total aerobic count in soil and water samples were clearly found. Presence of soil pathogens *B. cereus* and *L. monocytogenes* in soil is not uncommon considering their ubiquitous nature, however lower incidence of enterics is promising. No significant difference was observed on presence of bacterial pathogens with the climatic difference, though spatial difference could have had some effects in their presence. In general, conditions for survival of enteric pathogens once excreted from the animal are considered unfavorable which could be the cause of absence or lower incidence [Unc and Goss, 2004]. Various studies have previously reported low incidence of pathogens in vegetables at farm [Mukherjee *et al.*, 2004; Johnston *et al.*, 2005]. The present study was designed to provide an initial estimation on microbiological condition of agricultural products including environments (soil and water) based on collection of samples directly from farms. Some characteristic features of the study were random collection of samples, variety of vegetable and environmental samples and diversity of farms. To the best of our knowledge, this is the first study of its kind which provides information of microbial condition of vegetables, soil and water at farm level in Korea. The information gained from this study could help to determine the requirement for further studies into this area.

In conclusion, the result shows lower prevalence of pathogenic bacteria in vegetables, soil and water at the farm. Presence of bacterial pathogens like *B. cereus* and *L. monocytogenes* in soil is obvious, however lower presence of the enteric (*E. coli, Salmonella* spp.) shows excellent agricultural practice and manure management. Despite high food poisoning cases of *Salmonella* in Korea, its absence in agricultural products and environments indicates its low prevalence in fruits and vegetables, and hence low impact in food poisoning, compare to meat and meat products. Further increase of samples and sampling sites are needed to corroborate these results. Ultimately, educating farmers and workers with proper knowledge about good agriculture practices will help to lower the pathogen level at farm.

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