pISSN 1229-1153 *J. Fd Hyg. Safety*
Vol. 29, No. 1, pp. 1~5 (2014)
http://dx.doi.org/10.13103/JFHS.2014.29.1.001

Journal of Food Hygiene and Safety
Available online at http://www.foodhygiene.or.kr

Assessment of the Microbiological Quality of Vegetable from Urban Community Gardens in Korea

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(Received August 3, 2013/Revised December 2, 2013/Accepted March 10, 2014)

ABSTRACT - Many community gardens in large cities worldwide grow vegetables; however, no information regarding the levels of sanitary indicator bacteria and prevalence of foodborne pathogens in vegetables grown in urban community gardens is available. To evaluate the microbiological quality of vegetables from urban community gardens in Korea, 530 samples (nine types of vegetable, including Chinese cabbage, lettuce, radish leaves, spinach, mustard leaves, crown daisy, leek, Korean cabbage, and chicory) were collected at 11 urban community gardens in Seoul, Korea from September through October 2012. The levels of total aerobic bacteria, *Escherichia coli*, total coliforms, *Salmonella* spp. *Listeria monocytogenes*, and *E. coli* O157:H7 were evaluated quantitatively and/or qualitatively. The mean numbers of total aerobic bacteria and coliforms were 6.3 log CFU/g (range 3.8-8.1 log CFU/g) and 4.3 log CFU/g (range 2.1-6.4 log CFU/g), respectively. Total coliforms were detected on 67% of whole vegetables. Chicory showed the highest number of total aerobic bacteria and coliforms, whereas the lowest number of coliforms was detected on leeks. *E. coli* was detected on 2.3% of whole vegetables, including lettuce, radish leaves, mustard leaves, and chicory; however, foodborne pathogenic bacteria were not detected on any of the vegetable samples using this highly sensitive and validated procedure. Based on these findings, the presence of coliforms and *E. coli* demonstrates that opportunity for improvement of microbiological safety exists throughout the produce production chain, although no major foodborne pathogens were present in vegetables grown in urban community gardens.

Key words: Community garden, Vegetable, Microbial safety, Foodborne pathogens

Introduction

Sources of produce contamination include soil, feces, irrigation water, inadequately composted manure, wild and domestic animals, dirty equipment, and human handling. Fresh fruits and vegetables can be a vehicle for transmission of bacterial, parasitic, and viral pathogens that can cause human illness[1)]. Several reports have referred to raw vegetables harboring potential foodborne pathogens, including *Bacillus cereus, Clostridium perfringens*, *Salmonella* spp., *Escherichia coli* O157:H7 and *Listeria monocytogenes*[2-6)]. According to a report of foodborne disease outbreaks in the United States during 2008, the commodities associated with the most outbreak-related illnesses were fruits and nuts (1,755 illnesses) and vine-stalk vegetables (1,622), and the pathogen-commodity pair responsible for the most outbreaks was norovirus in leafy vegetables (18 outbreaks)[7)]. In Korea, the number of food-poisoning outbreaks associated with fruits and vegetables rose from 119 (4,577 patients) in 1998 to 271 (7,218 patients) in 2010[8)]. As the consumption of minimally processed vegetable produce rises, the importance of the food safety to public health concerns increases.

Community gardens are increasingly part of the urban fabric worldwide[9-10)]. These gardens, often built on underutilized land, are seen as having several positive health benefits. Additionally, many urban community gardens in Seoul, Korea have been created for citizens to improve their quality of life and enjoy their leisure time. Some reports focused on the health impact of urban community gardens[9-10)]; however, to our knowledge no microbiological assessment of vegetables grown in urban community gardens has been conducted. Here, to obtain baseline information regarding the occurrence of indicator bacteria (total aerobic bacteria, *E. coli*, and total coliforms) and bacterial foodborne pathogens

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(*Salmonella* spp. *L. monocytogenes* and *E. coli* O157:H7) in vegetables grown in urban community gardens in Seoul, a total of 530 samples (9 types of vegetable) were collected from 11 urban community gardens, and microbiological contamination levels and the presence of foodborne pathogens were analyzed using quantitative methods and qualitative techniques.

Materials and Methods

Collection of vegetable samples

Vegetable samples were obtained from 11 urban community gardens located in Seoul, Korea between September and October 2012. A total of 530 samples (nine types of vegetable: Chinese cabbage, lettuce, radish leaves, spinach, mustard leaves, crown daisy, leek, Korean cabbage, and chicory) were collected directly from fields and immediately transported to the laboratory without being washed. Microbial analyses were initiated within 6 h of sample collection.

Enumeration of total aerobic bacteria, *E. coli,* and coliforms

Samples were analyzed according to the Korea Food and Drug Administration Food Code (2008). Each vegetable sample (25 g) was weighed and mixed with 225 mL of sterilized peptone buffer. After homogenizing for 2 min at 230 rpm in a Stomacher machine (BagMixer, Interscience, Rockland, MA, USA), 1 mL of homogenized sample was serially diluted with 9 mL of sterilized peptone water. To calculate the total aerobic bacteria and coliforms, 1 mL of diluted sample was spread onto Petrifilm™ Aerobic Count Plates (3M, St Paul, MN, USA) and Petrifilm™ *E. coli*/ coliform plates (3M) under aseptic conditions, and plates were incubated for 24 h at 37ºC.

For detection of *E. coli*, 25-g samples were mixed with 225 mL of EC broth (Oxoid, Hampshire, England) and pre-incubated at 37ºC for 24 h. A loop of the pre-enriched culture was plated onto Eosin Methylene Blue agar (EMB, Oxoid) and then incubated at 37ºC for 24 h. The presence of *E. coli* was determined by the appearance of metallic green-colored colonies on the EMB plates.

Detection of foodborne pathogenic bacteria

Three subsamples (25 g each) were weighed and prepared for detection of *Salmonella* spp., *L. monocytogenes*, and *E. coli* O157:H7 according to the Korea Food and Drug Administration (2008) Food Code[11] and commercial immunoassays (BioSign™ antigen detection test, PBM, Princeton, NJ, USA).

For detection of *Salmonella* spp., samples (25 g each) were incubated in 225 mL of buffered peptone water, followed by incubation at 37ºC for 24 h. An aliquot (100 µL) was then transferred to 10 mL of Rappaport-Vassiliadis R10 broth (Difco Laboratories, Detroit, MI, USA) and incubated at 37ºC for 24 h. After incubation, samples were streaked on xylose lysine desoxycholate (Oxoid) and incubated at 35ºC for 24 h. Presumptive *Salmonella* isolates were confirmed using a BioSign™ *Salmonella* antigen detection kit (PBM) and VITEK-2 system (bioMérieux, Marcy l'Etoile, France).

For detection of *L. monocytogenes*, vegetable samples (25 g) were incubated in *Listeria* enrichment broth at 30ºC for 24 h, and 100 µL of cultured supernatant were transferred to Fraser Listeria broth (Difco Laboratories), and then incubated at 30ºC for 24 h. *Listeria* colonies are brown-green with a black halo on the media. When typical colonies were present, they were confirmed using a BioSign™ *Listeria* antigen detection kit (PBM), and using the VITEK-2 system.

For detection of *E. coli* O157:H7, vegetable samples (25 g) were incubated in 225 mL of mEC broth (Oxoid) at 37ºC for 24 h, and then transferred to sorbitol-MacConkey agar (Oxoid), supplemented with potassium tellurite and cefixime, and incubated at 37ºC for 24 h. When presumptive colonies were present, they were assayed using the BioSign™ *E. coli* O157 antigen detection kit (PBM) and identified using the VITEK-2 system.

The following *S. typhimurium, L. monocytogenes*, and *E. coli* O157:H strains were used as positive controls: ATCC 13314, ATCC 15313, and ATCC 43894 (ATCC, Manassas, VA, USA), respectively.

Statistical analysis

The data are presented as the means ± standard deviation (S.D.). Statistical significance was determined by ANOVA (StatView; Abacus Concepts Inc., Berkeley, CA, USA). Differences were deemed to be significant at *P*-values less than 0.05.

Results and Discussion

Community gardens are perceived by gardeners to exert psychological benefits through agricultural experience, produce fresh vegetables, and promote social health and community cohesion[9-10]. Baseline data regarding the microbiological quality of vegetables grown in urban community gardens located in major cities, including Seoul, Korea, are difficult to ascertain. Therefore, we evaluated the general microbiological qualities of such vegetables.

Aerobic colony count is an indicator of the overall microbial quality and shelf-life of a food product[12]. As shown in Table 1, the mean number of total aerobic bacteria was 6.3 log CFU/g, ranging from 3.8-8.1 log CFU/g. Chicory showed the highest mean number of total aerobic bacteria (6.9 log

Table 1. Total aerobic bacteria levels in nine types of vegetable collected from 11 urban community gardens in Seoul, Korea

Sample names	Numbers (%)	Samples in the indicated interval (%)[a]					Range[a]	Mean[a]
		< 5	5~6	6~7	7~8	> 8		
Chinese cabbage	80 (100.0)	14 (17.5)	54 (67.5)	9 (11.2)	2 (2.5)	1 (1.2)	4.3~8.1	5.7
Lettuce	100 (100.0)	17 (17.0)	62 (62.0)	13 (13.0)	8 (8.0)	0	4.8~7.8	5.8
Radish leaves	100 (100.0)	13 (13.0)	68 (68.0)	12 (12.0)	7 (7.0)	0	4.7~7.6	5.5
Spinach	50 (100.0)	8 (16.0)	27 (54.0)	12 (24.0)	3 (6.0)	0	4.3~7.6	6.1
Mustard leaves	40 (100.0)	13 (32.5)	19 (47.5)	7 (17.5)	1 (2.5)	0	4.2~7.3	5.3
Crown daisy	50 (100.0)	8 (16.0)	32 (64.0)	9 (18.0)	1 (2.0)	0	3.8~7.9	5.6
Leek	40 (100.0)	6 (15.0)	21 (52.5)	8 (20.0)	5 (12.5)	0	4.6~7.8	5.7
Korean cabbage	40 (100.0)	2 (5.0)	8 (20.0)	23 (57.5)	7 (17.5)		5.4~7.9	6.4
Chicory	30 (100.0)	0	6 (20.0)	15 (50.0)	9 (30.0)	0	5.3~7.6	6.9
Total	530 (100.0)	81 (15.3)	294 (56.0)	108 (20.4)	43 (8.1)	1 (0.2)	3.8~8.1	6.3

[a]Counts are expressed as log CFU/g of vegetable

Table 2. Total coliform levels in nine types of vegetable collected from 11 urban community gardens in Seoul, Korea

Sample names	Numbers (%)	Samples in the indicated interval (%)[a]					Range[a]	Mean[a]
		ND[b]	2~4	4~5	5~6	> 6		
Chinese cabbage	80 (100.0)	23 (28.8)	16 (20.0)	24 (30.0)	13 (16.3)	4 (5.0)	2.3~6.4	4.9
Lettuce	100 (100.0)	37 (37.0)	17 (17.0)	36 (36.0)	8 (8.0)	2 (2.0)	2.6~6.2	4.4
Radish leaves	100 (100.0)	24 (24.0)	18 (18.0)	35 (35.0)	22 (22.0)	1 (1.0)	2.7~6.3	4.7
Spinach	50 (100.0)	18 (36.0)	17 (34.0)	12 (24.0)	3 (6.0)	0	2.1~5.6	3.9
Mustard leaves	40 (100.0)	24 (60.0)	7 (17.5)	6 (15.0)	3 (7.5)	0	2.5~5.5	3.5
Crown daisy	50 (100.0)	8 (16.0)	22 (44.0)	19 (38.0)	1 (2.0)	0	2.9~5.4	4.3
Leek	40 (100.0)	22 (55.0)	9 (22.5)	6 (15.0)	3 (7.5)	0	2.7~5.9	3.4
Korean cabbage	40 (100.0)	8 (20.0)	8 (20.0)	16 (40.0)	6 (15.0)	2 (5.0)	2.4~6.3	4.8
Chicory	30 (100.0)	11 (36.7)	4 (13.3)	6 (20.0)	7 (23.3)	2 (6.7)	2.3~6.2	5.1
Total	530 (100.0)	175 (33.0)	118 (22.3)	160 (30.2)	66 (12.5)	11 (2.1)	2.1~6.4	4.3

[a]Counts are expressed as $\log_{10}$ CFU/g of vegetable
[b]ND : Not detected, > 10^2

CFU/g), while the lowest number was detected in mustard leaves (5.3 log CFU/g). Significant differences were noted between the numbers of total aerobic bacteria of chicory and mustard leaves ($P < 0.01$). Compared with previous reports, these data suggest similar levels of total aerobic bacteria on leafy green vegetables collected from both farms and retail stores[3-5,13]. It was reported that total aerobic bacteria levels ranged from 5.2-6.9 log CFU/g in several types of leafy greens and herbs, including green Swiss chard, turnip greens, collards, cabbage, kale, cilantro, and parsley at packing sheds[13]. In Korea, mean total aerobic bacteria counts of sesame leaf, dropwort, Chinese cabbage, Korean leek, lettuce, crown daisy, *Pimpinella brachycarpa*, and chicory from whole markets ranged from 2.5-9.4 log CFU/g[4] or 2.2×10^6 to 6.0×10^7 CFU/g[5].

Total coliforms and *E. coli* represent additional sanitary indicators and/or spoilage level indices. In the present study, coliforms were detected on 67% of whole vegetables, and the mean coliform count was 4.3 log CFU/g, ranging from 2.1-6.4 log CFU/g. Chicory showed the highest mean number of total coliforms (5.1 log CFU/g), and leek showed the lowest (3.4 log CFU/g) (Table 2); this difference was statistically significant ($P < 0.01$). The present results of total coliform counts were comparable to those reported elsewhere; i.e., 3.0-5.0 log CFU/g for fresh produce and minimally processed vegetables[4-6,13-15]. In Korea, mean coliform counts were 5.8 log CFU/g, and ranged from 1.0-7.8 log CFU/g from lettuce, sesame leaves, spinach, chicory, dropwort, crown daisy and fruit[4,16]. These studies indicated a higher contamination level than found in similar vegetables reported here. The variation observed may be attributed to methodological differences in the collection conditions of each study. In this investigation, samples were collected immediately from community gardens, thus minimizing additional sources of contamination. In contrast, the previous studies were performed with vegetables collected from markets[4,16], thereby increasing the opportunities for contamination.

We also evaluated *E. coli* contamination, which is currently the best-available indicator of fecal contamination[17]. *E. coli* was isolated from 2.3% of the samples, including chicory, radish leaves, mustard leaves, and lettuce. The mean number of *E. coli* detected was 2.6 log CFU/g, ranging from 2.1-

Table 3. *Escherichia coli* levels in nine types of vegetable collected from 11 urban community gardens in Seoul, Korea

Sample names	Numbers (%)	Samples in the indicated interval (%)[a]					Range[a]	Mean[a]
		ND[b]	2~3	3~4	4~5	> 5		
Chinese cabbage	80 (100.0)	80 (100.0)	0	0	0	0		
Lettuce	100 (100.0)	98 (98.0)	2 (2.0)	0	0	0	2.3~2.5	2.4
Radish leaves	100 (100.0)	96 (96.0)	3 (3.0)	1 (1.0)	0	0	2.2~3.4	2.6
Spinach	50 (100.0)	100 (100.0)	0	0	0	0		
Mustard leaves	40 (100.0)	38 (95.0)	1 (2.5)	1 (2.5)	0	0	2.3~3.6	2.9
Crown daisy	50 (100.0)	100 (100.0)	0	0	0	0	0	0
Leek	40 (100.0)	100 (100.0)	0	0	0	0	0	0
Korean cabbage	40 (100.0)	100 (100.0)	0	0	0	0	0	0
Chicory	30 (100.0)	26 (86.6)	2 (6.6)	1 (3.3)	1 (3.3)	0	2.1~4.2	3.1
Total	530 (100.0)	518 (97.7)	8 (1.5)	3 (0.6)	1 (0.2)	0	2.1~4.2	2.6

[a]Counts are expressed as $\log_{10}$ CFU/g of vegetable
[b]ND : Not detected, > 10^2

4.2 log CFU/g; the sample positivity percentages ranged from 2.0% for lettuce to 13.4% for chicory (Table 3). These findings are consistent with the results of Sant'Ana et al[6], who reported a 2.7% *E. coli* contamination rate in minimally processed vegetables at retail stores. However, a 2009 Alberta, Canada study found that 8.2% of fresh produce, including lettuce, spinach, carrots, and green onions were positive for *E. coli*[18], and *E. coli* was detected in 12.5 and 22.2% of conventional and organic lettuce, respectively[15]. In Korea, *E. coli* was isolated in 2.8% (ranging from 3.1-19.1%) of vegetables and fruits[4], which was higher than our results. In the present study, the levels of total aerobic bacteria, coliforms, and *E. coli* were relatively low and similar to those in other reports. The large variation in counts between studies could be due to differences in microbiological methodologies, the types of produce tested, source of samples and/or geographical locations. Importantly, these sanitary indices usually increase from the field through packing; thus, each step from production to consumption reinforces the importance of prevention of contamination and/or bacterial growth to ensure a high-quality product[19].

Vegetable crops produced in a natural environment cannot be expected to remain free from microbial agents, and the stages of fresh-cut-product processing, such as handling, cutting, shredding, slicing, and grating, are all potential sources of contamination that could further increase the microbial load[19]. Outbreaks of foodborne illnesses related to consumption of fresh produce have been documented by many authors[2,6,14,18]. In the present study, we also evaluated the prevalence of common foodborne pathogens that have been associated with vegetables using double-check systems, such as media culture systems and commercial immunoassay kits. All vegetables collected at 11 urban community gardens in Seoul had undetectable foodborne pathogens levels, possibly because the urban gardens were minimally contaminated by feces from wild animals and cattle due to the use of composted commercial organic fertilizer. Additionally, vegetables were harvested directly from farms, so no additional contamination occurred during vegetable processing. In recent reports, no *Salmonella*, *L. monocytogenes*, or *E. coli* O157:H7 were detected in lettuce, spinach, tomatoes, carrots, green onions, strawberries, sesame leaf, dropwort, Chinese cabbage, Korean leek, crown daisy, and chicory from markets or farms in Japan[14], Canada[18], and Korea[5,16].

The microbiological quality of fresh produce is a concern not only from a food safety perspective, but also because of losses due to the shorter product shelf-life. A community gardening program can reduce food insecurity, improve dietary intake, and strengthen family relationships[10]. This is the first study to provide baseline information regarding the occurrence and levels of microbiological indicators and common foodborne pathogens in selected produce items from urban community gardens in a major city. No foodborne pathogens were detected, although coliforms and *E. coli* were detected in 67.0 and 2.3% of the samples, respectively. The microbial qualities of the examined vegetables were similar to those reported elsewhere. However, the presence of coliforms and *E. coli* demonstrates that opportunity for improvement of microbiological safety exists throughout the produce production chain.

요 약

도시 텃밭에서 재배되는 농산물의 대부분은 식탁에 바로 오르는 채소류로 안전성 확보가 요구되나 아직 이에 대한 자료가 없다. 본 연구는 도시 텃밭에서 재배되는 채소류의 생물학적 안전성을 평가하기 위한 기초 자료를 확보하기 위하여, 2012년 9월부터 10월 사이에 서울시내 11곳의 도시텃밭에서 채취한 9종 채소류(배추, 상추, 무잎,

시금치, 겨자잎, 쑥갓, 파, 양배추와 치커리) 530 표본을 대상으로 총호기성균(일반세균), 대장균/대장균군, *Salmonella* spp. *Listeria monocytogenes* 및 *E. coli* O157:H7 오염 수준을 정량적으로 혹은 정성적으로 평가하였다.

조사한 농산물에 존재하는 총호기성 세균과 대장균군의 평균 수는 각각 6.3 log CFU/g (범위, 3.8-8.1 log CFU/g) 및 4.3 log CFU/g (범위, 2.1-6.4 log CFU/g) 이었다. 대장균군은 전체 표본의 67%에서 발견되었다. 치커리에서 가장 많은 수의 총호기성 세균과 대장균군이 검출되었고, 부추에서 가장 적은 수의 대장균군 오염을 보였다. 대장균은 전체 채소류의 2.3%에서 발견되었으나, 농산물별로는 상추, 무잎, 갓과 치커리에서 발견되었다. 그렇지만, 조사한 농산물에서 식중독 유발 세균인 *Salmonella* spp., *L. monocytogenes* 및 *E. coli* O157:H7은 검출되지 않았다. 이상의 결과로 보아, 서울시내 도시텃밭에서 재배되는 채소류에서 식중독 유발 세균은 발견되지 않았으나, 재배 과정중 대장균군 및 대장균의 오염을 줄이기 위한 노력이 요구된다.

Acknowledgements

The present research has been performed according to a planning project of Korea Institute of Planning and Evaluation for Technology in Food, Agriculture, Forestry, and Fisheries (ARPC, Project No. 608010-5) and the support of Rural Development Administration, Republic of Korea (Project No. PJ9071142012).

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