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Soli, Kevin Webby
Laboratory of Food Hygienic Chemistry, Division of Food Science and Biotechnology, Department of Bioscience and Biotechnology, Graduate School of Bioresource and Bioenvironmental Sciences, Kyushu University

Yoshizumi, Asako
Laboratory of Food Hygienic Chemistry, Division of Food Science and Biotechnology, Department of Bioscience and Biotechnology, Graduate School of Bioresource and Bioenvironmental Sciences, Kyushu University

Yamakawa, Mami
Laboratory of Food Hygienic Chemistry, Division of Food Science and Biotechnology, Department of Bioscience and Biotechnology, Graduate School of Bioresource and Bioenvironmental Sciences, Kyushu University

Mishima, Tomoko
Laboratory of Food Hygienic Chemistry, Division of Food Science and Biotechnology, Department of Bioscience and Biotechnology, Graduate School of Bioresource and Bioenvironmental Sciences, Kyushu University

他

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Bacterial Contamination and Microflora of Several Fresh Produce

Kevin Webby SOLI[1], Asako YOSHIZUMI[1], Mami YAMAKAWA[1], Tomoko MISHIMA[1], Ken–ichi HONJOH and Takahisa MIYAMOTO*

Laboratory of Food Hygienic Chemistry, Division of Food Science and Biotechnology,
Department of Bioscience and Biotechnology, Faculty of Agriculture,
Kyushu University, Fukuoka 812–8581, Japan
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Viable counts were enumerated in 36 raw samples of 19 different vegetables. Coliform, fecal coliform, and *E. coli* were determined in 31 vegetable samples. Tomato was found to have the lowest viable count of 2.12 log cfu/g while radish sprout had the highest count of 9.05 log cfu/g. Although *E. coli* was not detected in all the vegetables tested, most of these vegetables were positive for fecal coliform. Viable counts of the tenth leaves from the outside were lower by only 1 log cfu/g than that of the outermost leaves in cabbage and lettuce. Among viable counts of vegetable parts, celery leaves, lower stems in radish sprout, and spinach were found to have the highest viable counts of 7.28 log cfu/g, 9.27 log cfu/g, and 6.10 log cfu/g respectively while lower stem in parsley had the lowest count of 5.10 log cfu/g. The microflora of the four vegetables, celery, parsley, radish, and radish sprout were determined by using biochemical methods. There were 105, 50, 48, 130, and 61 bacterial isolates from celery, lettuce, parsley, radish, and radish sprout, respectively. The predominant bacterium on the four vegetables was about 30–60% Gram–negative *Flavobacterium* or *Xanthomonas*. Other Gram–negative bacteria isolated from the vegetables include 11% *Neisseria* or *Veillnella* (celery), 18% *Moraxella* (radish), 15% *Alcaligenes* and 12% *Pseudomonas* (radish sprout) while *Enterobacteriaceae* accounted for less than 5% for each of the flora of celery, parsley, and radish sprout. On the contrary, parsley had 25% *Kurthia* or *Bacillus*, and 13% *Micrococcus*, both Gram–positive.

INTRODUCTION

Consumption of fresh produce has increased, mainly because of heightened awareness of the benefits of a healthy diet. Leafy or salad vegetables are mainly eaten fresh and have become increasingly popular because of their high sensory quality and convenience (Lund, 1989). These vegetables are high in water, vitamins, minerals, and dietary fiber (Weerakkody, 2003). Minimal processing to which fresh produce is subjected renders vegetables to physiological deterioration, biochemical changes and microbial degradation of product (O'Beirne and Francis, 2003). Additionally, cut tissues release nutrients that support the growth of microflora present on raw produce (Li *et al.*, 2001). Ready–to–use vegetables harbor large and diverse populations of microorganisms, and counts of 5.0–7.0 log cfu/g are frequently present. Eighty to ninety percent of bacteria are Gram–negative rods, predominantly *Pseudomonas, Enterobacter* or *Erwinia* species as reviewed by Francis *et al.* (1999). Fresh–cut vegetables retain much of their indigenous microflora after minimal processing, however, pathogens may form part of this microflora, posing a potential food safety problem (Francis, *et al.*, 1999). Surveys of bagged salads often recover low numbers of enteropathogens (Szabo *et al.*, 2000; Francis *et al.*, 1999; Lin *et al.*, 1996). According to the study of the disease risks of food groups by Adak *et al.* (2005), a low risk ratio for salad vegetables was reported even though fresh produce has a considerable impact on disease incidence in a population. A factor that impacts microbial quality is the increase in importation, which aims to meet consumer demands for a wide choice of exotic fruit and vegetables year–round. As hygiene standards of irrigation, at harvest, and during storage can vary widely in different countries, the potential for contamination of produce may increase. The objectives of this study are to determine the viable counts and the regions of natural bacterial contamination in various vegetables, and identify the microflora of celery, lettuce, parsley, radish, and radish sprouts that are distributed in Fukuoka area, using biochemical methods.

MATERIALS AND METHODS

Preparation of vegetables

Fresh vegetables used in this study were purchased from a local supermarket in Fukuoka, Japan, stored at 6 ℃ and used in experiments within 24 h. The vegetables were manually trimmed to remove damaged leaves and stems, or cores of lettuce and cabbage, and briefly washed with deionized water to remove soil, or dirt.

Measurement of viable counts, coliform, fecal coliform and *E. coli*

Excess water on vegetable leaves was removed before 25 g of each sample leaves was weighed into stomacher bags containing filter mesh. A 10–fold dilution was made after 225 mL of peptone water was added aseptically to the bag in a biosafety cabinet. The sample was homogenized for 1 min using a Masticator (IUL instruments, Barcelona, Spain). Filtrate of the homogenate was serially diluted into required concentrations. Aliquots of

[1] Laboratory of Food Hygienic Chemistry, Division of Food Science and Biotechnology, Department of Bioscience and Biotechnology, Graduate School of Bioresource and Bioenvironmental Sciences, Kyushu University

* Corresponding author (E–mail: tmiyamot@agr.kyushu–u.ac.jp)

0.1 mL were surface spread onto tryptic soy agar (TSA, Becton Dickinson) in duplicates and incubated at 25 °C for 72 h for enumeration of total aerobic mesophilic microorganisms (viable count). For enumeration of viable counts of each of the leaves of cabbage, and lettuce, single leaves were weighed and added into stomacher bags and diluted 10–folds with sterile water and homogenized for 30 s. For viable counts of parts of other vegetables, each plant was aseptically cut into three parts (leaf, upper and lower stems), weighed and diluted 10–folds with sterile water and homogenized for 30 s. Coliform bacteria, fecal coliform, and *E. coli* were measured both qualitatively and quantitatively (3–tube MPN method) according to the standard method described by Pharmaceutical Society of Japan (2010). After the enumeration of viable cells, 50–200 colonies cultivated in the same plate were isolated for identification by biochemical tests.

Identification of microflora

General biochemical tests (Gibbs and Skinner, 1966) were carried out to identify genus of the microflora of vegetables based on culturable cells.

RESULTS

Viable, coliform, and *E. coli* counts of various vegetables

Viable counts of 36 raw samples of 19 different vegetables were measured. Coliform, fecal coliform, and *E. coli* were determined in 31 vegetable samples. Table 1 shows the viable, coliform, fecal coliform, and *E. coli* counts of various vegetables commercially distributed in Fukuoka. The viable counts were distributed widely among the samples from 2.12 log cfu/g (tomato) to 9.05 log cfu/g (radish sprout). Although *E. coli* was not

Table 1. Viable, coliform, fecal coliform and *E. coli* counts of commercially available vegetables

Vegetables	viable counts (log cfu/g)	coliform (log cfu/10 g)	fecal coliform (log cfu/10 g)	*E.coli* (log cfu/10 g)
Asparagus	4.78	>3.15	+	–
Broccoli	2.80	0.56	–	–
Cabbage 1	2.48	1.36	+	–
Cabbage 2	5.73	>3.15	–	–
Celery	7.03	1.97	0.56	–
Cucumber 1	5.92	>3.15	+	–
Cucumber 2	4.77	1.63	+	–
Cucumber 3	5.93	>3.15	+	–
Eggplant 1	4.70	>3.15	+	–
Eggplant 2	4.95	2.38	+	–
Eggplant 3	4.79	>3.15	0.56	–
Eggplant 4	5.03	>3.15	+	–
Eggplant 5	4.75	>3.15	–	–
Ginger	3.57	ND	ND	ND
Japanese ginger	7.36	ND	ND	ND
Komatsuna 1	5.93	1.97	+	–
Komatsuna 2	6.62	>3.15	+	–
Leaf lettuce 1	5.92	>3.15	+	–
Leaf lettuce 2	6.03	3.04	+	–
Leek 1	5.50	>3.15	+	–
Leek 2	4.77	2.66	+	–
Lettuce 1	6.24	>3.15	–	–
Lettuce 2	2.98	2.66	+	–
Mitsuba	6.25	>3.15	–	–
Oil–seed rape	5.79	3.04	+	–
Perilla	7.12	ND	ND	ND
Parsley	6.36	ND	ND	ND
Radish sprout	9.05	ND	ND	ND
Spinach 1	6.80	>3.15	+	–
Spinach 2	7.95	>3.15	+	–
Spinach 3	6.66	>3.15	+	–
Tomato 1	2.67	2.66	+	–
Tomato 2	4.31	2.38	+	–
Tomato 3	5.24	2.66	+	–
Tomato 4	3.57	1.63	+	–
Tomato 5	2.12	1.97	+	–

–, Negative
+, Positive
ND, Not done

Table 2. Viable counts of each leaf of cabbage and lettuce

Vegetables	Viable counts (log cfu/g) of leaf									
	Outermost →									Inner
	1	2	3	4	5	6	7	8	9	10
Cabbage	6.49	6.55	6.63	6.58	6.11	6.37	5.47	5.51	5.56	5.46
Lettuce	7.26	7.17	7.18	7.12	7.04	6.92	6.50	6.38	6.18	6.07

detected in all the vegetables tested, most of these vegetables were positive for fecal coliform, suggesting that almost all of the vegetables were spotted with soil or water contaminated with animal feces recently. Coliform counts differed from one sample to another even with the same vegetable. The counts were relatively high in cucumber, eggplant, leek, lettuce, oil–seed rape, spinach, and tomato.

Viable counts of parts of vegetables

Viable counts of each leaf of cabbage and lettuce were measured and the results are shown in Table 2. Counts of the tenth leaves were lower than those of the outermost leaves by only 1 log cfu/g in cabbage and lettuce. Outer leaves and surface of fresh produce are inevitably exposed to various handling processes before and after harvest, therefore affecting the viable counts of produce.

Viable counts of the leaf, upper stem, and lower stem of celery, parsley, radish sprout, and spinach were measured and count of each part is shown in Table 3. Celery leaves, lower stems in radish sprout, and spinach were found to have the highest viable counts of 7.28 log cfu/g, 9.27 log cfu/g, and 6.10 log cfu/g respectively while lower stem in parsley had the lowest count of 5.10 log cfu/g.

Table 3. Viable counts of parts of some vegetables

Vegetables	Viable counts (log cfu/g) of each part		
	leaf	upper stem	lower stem
Celery	7.28	5.89	5.88
Parsley	6.36	6.36	5.10
Radish sprout	8.96	8.50	9.27
Spinach	5.96	5.13	6.10

Microflora of some vegetables

Microflora of celery, lettuce, parsley, radish, and radish sprout were determined by biochemical tests and the ratio (%) of the identified genera are shown in Table 4. There were 105 bacterial colonies with a viable count of 7.03 log cfu/g from celery, 50 colonies (6.24 log cfu/g) from lettuce, 48 colonies (6.36 log cfu/g) from parsley, 61 colonies (5.22 log cfu/g) from radish, and 130 colonies (8.73 log cfu/g) from radish sprout that were isolated and identified respectively.

The predominant bacterium on celery was 30% *Flavobacterium* or *Xanthomonas* followed by 11% *Neisseria* or *Veillonella* while each of the remaining bacteria was less than 10%. *Flavobacterium, Xanthomonas, Neisseria* and *Veillonella* are Gram–negative bacteria including *Enterobacteriaceae*, which accounted for 4.8% of the flora.

The predominant bacterium on lettuce was 82% *Flavobacterium* or *Xanthomonas* followed by 12% *Pseudomonas* and 2% *Neisseria* or *Veillonella*. *Flavobacterium, Xanthomonas, Neisseria, Veillonella*

Table 4. Microflora of some vegetables determined by biochemical tests

Genus of bacteria	Ratio (%)				
	Celery	Lettuce	Parsley	Radish	Radish sprout
Acinetobacter	1.9	0.0	0.0	1.6	2.3
Alcaligenes	2.9	0.0	2.1	3.3	14.6
Enterobacteriaceae	4.8	0.0	2.1	0.0	3.8
Flavobacterium or *Xanthomonas*	30.5	82.0	50.0	63.9	46.2
Kurthia or *Bacillus*	8.6	0.0	25.0	8.2	7.7
Lactobacillus	4.8	0.0	0.0	0.0	0.0
Micrococcus	8.6	0.0	12.5	0.0	1.5
Moraxella	8.6	0.0	2.1	18.0	7.7
Neisseria or *Veillonella*	11.4	2.0	2.1	1.6	0.8
Pseudomonas	6.7	12.0	2.1	3.3	11.5
Staphylococcus	1.9	0.0	0.0	0.0	0.0
Streptococcus	2.9	0.0	0.0	0.0	0.0
Vibrio	6.7	0.0	2.1	0.0	3.8
Others	0.0	4.0	0.0	0.0	0.0
Total	100.0	100.0	100.0	100.0	100.0
Number of isolates	105	50	48	61	130
Viable counts (log cfu/g)	7.03	6.24	6.36	5.22	8.73

and *Pseudomonas* are Gram–negative bacteria.

The predominant bacterium on parsley was 50% *Flavobacterium* or *Xanthomonas* followed by 25% *Kurthia* or *Bacillus*, 13% *Micrococcus*, while each of the remaining bacteria was less than 3%. *Kurthia, Bacillus*, and *Micrococcus* are Gram–positive bacteria. *Enterobacteriaceae* accounted for 2.1% of the flora.

The predominant bacterium on fresh radish was 64% *Flavobacterium* or *Xanthomonas* followed by 18% Gram–negative *Moraxella*, while each of the remaining bacteria was less than 10%.

The predominant bacterium on radish sprout was 46% *Flavobacterium* or *Xanthomonas* followed by 15% *Alcaligenes*, 12% *Pseudomonas*, while each of the remaining bacteria was less than 10%. *Enterobacteriaceae* accounted for 3.8% of the flora.

DISCUSSION

Vegetables harbor large and diverse populations of microorganisms, and counts of 5.0–7.0 log cfu/g are frequently present. Eighty to ninety percent of bacteria are Gram–negative rods, predominantly *Pseudomonas, Enterobacter* or *Erwinia* species as reviewed by Francis *et al*. (1999). Furthermore, Konishi *et al*. (2001) reported that the standard plate counts of vegetables are widely distributed from less than 1.0 to 8.0 log cfu/g, and in particular, the counts of alfalfa and white radish sprouts were high at 7.0 log cfu/g. In our study, radish sprout had the highest count of 9.05 log cfu/g among the 19 vegetables tested. It has been shown that a variety of foodborne pathogens are present in sprouts. *Salmonella* sp., *Listeria monocytogenes, Staphylococcus aureus, Bacillus cereus* and *Aeromonas hydrophila* have been isolated from sprouted seeds, such as alfalfa, cress, and soybean (Beuchat, 1996). *E. coli* O157 and *Salmonella* have been reported to be the causative agents of outbreaks of foodborne illness associated with sprouts (Jackson, 1998). Prior to sprouting, seeds are contaminated with significant levels of microorganisms. Various surveys have revealed viable counts of 4.48–4.78 log cfu/g (Andrews *et al*., 1979), and 5.95 log cfu/g (Andrews *et al*., 1982) on alfalfa seeds. Untreated or improperly treated water, animal waste or manure, poor sanitation of equipment and poor personal hygiene could be potential sources of contamination for seeds or sprouts at the sprouting facility (Beuchat, 1996). These contamination factors may have been the reason why the viable count was the highest in radish sprout.

Jacques *et al*. (1994) observed that there were greater bacterial population densities on outer leaves than on inner leaves of endive (salad vegetable) throughout the growing season. The differences were statistically significant for total bacterial populations at all sampling times and were often significant for fluorescent and pectolytic bacterial populations. We have also observed that the viable counts of the tenth leaves of both cabbage and lettuce were lower by only 1 log cfu/g than that of the outermost leaves. Celery leaves, lower stems in radish sprout, and spinach were found to have the highest viable counts of 7.28 log cfu/g, 9.27 log cfu/g, and 6.10 log cfu/g respectively while lower stem in parsley had the lowest count of 5.10 log cfu/g.

There were 105, 50, 48, 130, and 61 bacterial isolates from celery, lettuce, parsley, radish, and radish sprout, respectively. The predominant bacterium on all of the vegetables was about 30–60% Gram–negative *Flavobacterium* or *Xanthomonas*. Other Gram–negative bacteria isolated from the vegetables include 11% *Neisseria* or *Veillnella* (celery), 18% *Moraxella* (radish), 15% *Alcaligenes* and 12% *Pseudomonas* (lettuce and radish sprout). On the contrary, parsley had 25% *Kurthia* or *Bacillus* and 13% *Micrococcus*, both Gram–positive. *Enterobacteriaceae* was detected from celery, parsley, and radish sprout, suggesting the possibility of contamination with coliform, fecal coliform, *E. coli* or other pathogenic bacteria.

There is little data about interactions between pathogens and indigenous microflora of vegetable products (Francis *et al*., 1999). Knowing the microflora of vegetables will help us clarify the changes of microflora of different fresh produce after exposure to decontamination treatment that is aimed to ensure quality and prolong shelf–life of fresh produce.

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