Research Note

Microbiological Examination of Vegetable Seed Sprouts in Korea

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ABSTRACT

Sprouted vegetable seeds used as food have been implicated as sources of outbreaks of Salmonella and Escherichia coli O157:H7 infections. We profiled the microbiological quality of sprouts and seeds sold at retail shops in Seoul, Korea. Ninety samples of radish sprouts and mixed sprouts purchased at department stores, supermarkets, and traditional markets and 96 samples of radish, alfalfa, and turnip seeds purchased from online stores were analyzed to determine the number of total aerobic bacteria (TAB) and molds or yeasts (MY) and the incidence of Salmonella, E. coli O157:H7, and Enterobacter sakazakii. Significantly higher numbers of TAB (7.52 log CFU/g) and MY (7.36 log CFU/g) were present on mixed sprouts than on radish sprouts (6.97 and 6.50 CFU/g, respectively). Populations of TAB and MY on the sprouts were not significantly affected by location of purchase. Radish seeds contained TAB and MY populations of 4.08 and 2.42 log CFU/g, respectively, whereas populations of TAB were only 2.54 to 2.84 log CFU/g and populations of MY were 0.82 to 1.69 log CFU/g on alfalfa and turnip seeds, respectively. Salmonella and E. coli O157:H7 were not detected on any of the sprout and seed samples tested. E. sakazakii was not found on seeds, but 13.3% of the mixed sprout samples contained this potentially pathogenic bacterium.

Consumption of sprouted vegetable seeds in Korea has been increasing due in part to their nutritional benefits. Since sprouts are often eaten without being heated or cooked, they have occasionally been implicated in foodborne diseases such as Escherichia coli O157:H7 infections and salmonellosis (26). Sprouts caused at least 37 outbreaks of foodborne disease in several countries between 1995 and 2005 (10). Growth of Salmonella and E. coli O157:H7 that may be present on seeds or introduced from the environment during sprouting and subsequent handling may occur. Enterobacter sakazakii is known to grow on several types of fresh-cut fruits and vegetables (12), but its incidence and behavior on seed sprouts have not been described.

Microbial surveys have shown that sprouts contain high populations of total aerobic bacteria (TAB) or mesophilic aerobic bacteria (1, 9, 14, 17, 19, 20, 23, 28). However, the incidence of Salmonella or E. coli O157:H7 on sprouts varies. Robertson et al. (21), Warriner et al. (28), and Abadias et al. (1) did not detect Salmonella or E. coli O157:H7 on sprouts, whereas 23 to 94% of test samples were positive for the presence of Salmonella in other studies (9, 23).

The general microbiological quality of soybean sprouts commonly consumed in Korea has been investigated previously (13, 18). However, there is limited information on the microbiological quality of other sprouts. Therefore, the objective of this study was to determine the general microbiological quality and the prevalence of Salmonella, E. coli O157:H7, and E. sakazakii in different types of sprouts and seeds commercially available for consumers in Korea.

MATERIALS AND METHODS

Sprout samples. Forty-five samples of packaged radish (Raphanus sativus) sprouts and 45 packages of mixed sprouts were purchased from five department stores, five supermarkets, and five traditional markets in Seoul, Korea, in May and June 2007. Three subsamples from each store or market were analyzed. The mixed sprouts contained at least three seed types, including clover (Trifolium sp.), alfalfa (Medicago sativa), Brussels sprouts (Brassica oleracea Gemmifera Group), broccoli (Brassica oleracea), kohlrabi (Brassica oleracea Gongylodes Group), red clover (Trifolium pratense), and Chinese cabbage (Brassica rapa subsp.).

Seed samples. Thirty-three samples of radish (R. sativus) seeds, 36 samples of alfalfa (M. sativa) seeds, and 27 samples of turnip (Brassica rapa var. rapa) seeds were purchased from nine online stores in Korea in September and October 2007. Triplicate subsamples of each sample were analyzed.

Numbers of TAB and MY. Sterile saline (100 ml) was added to a 10-g sprout or seed sample in a 400-ml Polyolefin bag (400 ml; Interscience, St Nom La Breteche, France). The sample and saline were homogenized using a bag mixer (Interscience) for 1 min and then serially diluted in sterile 0.1% peptone water.Undiluted (0.25 ml in quadruplicate and 0.1 ml in duplicate) and diluted samples (0.1 ml in duplicate) were spread plated on plate count agar (Difco, Becton Dickinson, Sparks, MD) and dichloran

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TABLE 1. Populations of TAB and MY and presence of Salmonella, E. coli O157:H7, and E. sakazakii on radish sprouts and mixed sprouts

<table>
<thead>
<tr>
<th>Type of sprout</th>
<th>Location of purchase</th>
<th>No. of samples</th>
<th>Population of microorganisms (log CFU/g)</th>
<th>No. (%) of samples positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mixed</td>
<td>Department store</td>
<td>15</td>
<td>B 6.93 AB, B 6.41 A</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Mixed</td>
<td>Supermarket</td>
<td>15</td>
<td>B 6.82 B, B 6.42 A</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Mixed</td>
<td>Traditional market</td>
<td>15</td>
<td>B 7.13 A, B 6.68 A</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Mixed</td>
<td>All locations</td>
<td>45</td>
<td>B 6.97, B 6.50</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Mixed</td>
<td>Department store</td>
<td>15</td>
<td>A 7.35 B, A 7.25 A</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Mixed</td>
<td>Supermarket</td>
<td>15</td>
<td>A 7.64 A, A 7.37 A</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Mixed</td>
<td>Traditional market</td>
<td>15</td>
<td>A 7.57 A, A 7.49 A</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Mixed</td>
<td>All locations</td>
<td>45</td>
<td>A 7.52, A 7.36</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

*Within the same type of sprout, mean values in the same column that are followed by the same letter are not significantly different (P ≥ 0.05). Within the same location of purchase, mean values in the same column that are preceded by the same letter are not significantly different (P ≥ 0.05). The detection limit was 1.0 log CFU/g (10 CFU/g).

*The detection limit was 1 CFU/25 g.

*Values represent data collected from three locations.

rose bengal chloramphenicol (Difco, Becton Dickinson) agar for enumeration of TAB and molds or yeasts (MY), respectively. The plate count agar plates were incubated at 37°C for 48 h, and di-chloran rose bengal chloramphenicol agar plates were incubated at 25°C for 5 days before colonies were counted.

**Presence of Salmonella.** Sprout or seed samples (25 g) and 225 ml of sterile 1% peptone water were placed in a 400-ml Polyolefin (Interscience) bag, mixed, and incubated at 35°C for 20 h for preenrichment. A 0.1-ml aliquot of the preenriched mixture was added to 10 ml of Rappaport-Vassiliadis enrichment broth (Difco, Becton Dickinson) and incubated at 42°C for 24 h for recovery of *Salmonella.* The enrichment mixture was streaked onto MacConkey agar (Difco, Becton Dickinson), and plates were incubated at 35°C for 24 h. Cells from presumptive *Salmonella* colonies formed on MacConkey agar were subjected to confirmation tests using the API 20E kit (BioMérieux Vitek, Inc., Hazelwood, MO).

**Presence of E. coli O157:H7.** Modified EC (Difco, Becton Dickinson) broth (225 ml) was combined with a 25-g sprout or seed sample in a 400-ml Polyolefin bag and incubated at 35°C for 24 h for enrichment of *E. coli O157:H7.* The enrichment mixture was streaked onto MacConkey sorbitol agar (BD/Difco) and incubated at 35°C for 18 to 20 h. Colonies presumptive for *E. coli O157:H7* on the MacConkey sorbitol agar plates were streaked onto eosin methylene blue (Difco, Becton Dickinson) agar and incubated at 35°C for 24 h. Metallic green colonies formed on eosin methylene blue agar were transferred to tryptic soy agar (TSA; Difco, Becton Dickinson) and incubated at 35°C for 24 h. Cells from colonies formed on TSA were Gram stained and observed using a light microscope. Only cells from colonies determined to be gram negative were subjected to confirmation as *E. coli O157:H7* by using the API 20E kit.

**Presence of E. sakazakii.** A 25-g sprout or seed sample was placed in a 400-ml bag containing 225 ml of *Enterobacteriaceae* enrichment broth (Difco, Becton Dickinson) and incubated at 37°C for 24 h. The *Enterobacteriaceae* enrichment broth was streaked onto violet red bile glucose agar (Difco, Becton Dickinson) and incubated at 37°C for 16 to 18 h. Cells from presumptive *E. sakazakii* colonies were streaked on TSA and incubated at 25°C for 16 to 18 h. Cells from yellow-pigmented colonies were subjected to confirmation using the API 20E kit. Simultaneously, 16S rRNA gene sequencing of cells from presumptive *E. sakazakii* colonies was performed by Macrogen Inc., Seoul, Korea (http://www.macrogen.com). The 16S rRNA gene sequences were analyzed by an NCBI BLAST search (http://www.ncbi.nlm.nih.gov/blast/Blast.cgi) for identification of *E. sakazakii.*

**Statistical analysis.** All sampling was performed in triplicate using sprouts purchased from five different lots and seeds purchased from 9 to 11 different lots. Data were analyzed using the general linear model of the Statistical Analysis Systems procedure (SAS Institute, Cary, NC). Fisher’s least significant difference test was used to determine if the numbers of TAB and MY detected in the sprouts and seeds were significantly affected by purchase location and type of sprout or seed. Significant differences are presented at a 95% confidence level (P ≤ 0.05).

**RESULTS AND DISCUSSION**

**Microorganisms on sprouts.** Populations of TAB and MY detected on radish sprouts and mixed sprouts purchased at various retail locations are shown in Table 1. All sprout samples contained TAB at ≥6.82 log CFU/g. Mixed sprouts contained significantly higher numbers of TAB than did radish sprouts. Similar populations of TAB on sprouts have been observed in other studies. Aerobic plate counts of approximately 7.0 to 9.0 log CFU/g of mixed sprouts and 7.15 to 8.15 log CFU/g of alfalfa, clover, sunflower, mung bean, and broccoli sprouts were reported by Prokopowich and Blank (19) and Matos et al. (17), respectively. Broccoli and mung bean sprouts had TAB counts of >6.0 log CFU/g (20) and 7.4 to 8.4 log CFU/g (28). Fifteen samples of soybean and alfalfa sprouts contained aerobic mesophilic microorganisms at a population of 7.9 log CFU/g and psychrotrophic microorganisms at 7.3 log CFU/g (1). Saroj et al. (23) analyzed mung, matki, chana, and vatana sprouts for aerobic plate counts and found 7.6 to 8.9 log CFU/g. Soybean sprouts purchased in Korea contained 7.7 to 8.1 log CFU/g of mesophilic microorganisms and 5.1 to 8.7 log CFU/g of psychrotrophic microorganisms (13). These large microbial populations raise concerns...
about potential risks of foodborne diseases resulting from the occasional presence of pathogens.

Results from our survey indicate that the general microbiological quality of sprouts was not correlated with the location of purchase. Choi et al. (4) also found no significant differences in the numbers of TAB on three types of fresh vegetables purchased from traditional markets and a supermarket in Korea.

Numbers of MY were significantly higher on mixed sprouts (7.25 to 7.49 log CFU/g) than on radish sprouts (6.41 to 6.68 log CFU/g) (Table 1). Similar to the TAB counts, the number of MY on sprouts was not affected by the location of purchase. Others have reported large variations in the number of MY on sprouts. Mung bean sprouts collected in the Philippines were reported to contain MY at 1.23 to 5.90 log CFU/g (9). Abadias et al. (1) reported MY counts of ca. 5 log CFU/g on soybean and alfalfa sprouts in Spain. In the United States, Tournas (27) detected MY at ca. 7, 5, and 5 log CFU/g on bean, alfalfa, and broccoli sprouts, respectively. Yeasts were more predominant than molds, although the percentages varied (57.1 to 96.3%), depending on the type of sprout.

Radish sprouts and mixed sprouts were analyzed for the presence of Salmonella, E. coli O157:H7, and E. sakazakii (Table 1). The major pathogens causing foodborne diseases associated with consumption of sprouts are Salmonella and E. coli O157:H7. These pathogens were not detected in sprout samples analyzed in this study. Abadias et al. (1) also failed to isolate Salmonella from soybean and alfalfa sprouts in Spain. Robertson et al. (21) did not detect Salmonella and E. coli O157:H7 in alfalfa, mung bean, radish, or mixed sprouts in Norway, and Warriner et al. (28) did not detect these pathogens in mung bean sprouts in the United Kingdom. On the other hand, Salmonella was isolated from 94% of mung bean sprouts tested in the Philippines (9). In India, Saroj et al. (23) found that 23% of mung bean sprouts harbored Salmonella but E. coli O157: H7 was not isolated from the test samples. Enterohemorrhagic E. coli, E. coli O157, and Salmonella were found by PCR analysis in 6.0, 1.5, and 7.0%, respectively, of 200 samples of sprouts collected in Seattle, WA (22). Loui et al. (15) analyzed four brands of alfalfa sprouts purchased at grocery stores in the United States and determined that 1.3 and 2.2% of two brands were positive for Salmonella.

We were interested in analyzing sprouts and seeds for the presence of E. sakazakii because of its widespread occurrence in the environment, making it a potential contaminant of vegetables (11), seeds, and sprouts. High numbers of E. sakazakii in sprouts could lead to infections in immunocompromised adults. Fatalities in elderly immunocompromised adults infected with E. sakazakii have been reported (3, 5). Presumptive E. sakazakii colonies obtained from radish and mixed sprouts were analyzed by 16S rRNA gene sequencing to confirm their identities. The presumptive E. sakazakii colonies from radish sprouts did not match E. sakazakii, whereas six isolates from mixed sprouts had 99% identity to 16S rRNA sequences of E. sakazakii strains, either ATCC BAA-894 or V328 (7). According to these results, 13.3% of the mixed sprout samples were positive for E. sakazakii. Other studies have also shown the presence of E. sakazakii in sprouts, e.g., alfalfa sprouts (6). Most of the coliform bacteria detected by Robertson et al. (21) in alfalfa, mung bean, radish, and mixed sprouts were identified as Enterobacter cloacae, E. sakazakii, and Klebsiella pneumoniae subsp. pneumoniae.

Microorganisms on seeds. Table 2 shows numbers of TAB and MY present on radish, alfalfa, and turnip seeds. The largest TAB population (4.08 log CFU/g) was detected on radish seeds, while alfalfa and turnip seeds contained TAB at populations of 2.54 and 2.84 log CFU/g, respectively. Soylemez et al. (24) reported aerobic plate counts of approximately 4 log CFU/g for alfalfa seeds. Relatively low numbers of microorganisms on seeds were observed on seeds compared to numbers of TAB on sprouts. Large populations of microorganisms on sprouts can be attributed largely to favorable growth conditions such as water activity and temperature during sprouting. Numerous studies have demonstrated that microorganisms grow exponentially on seeds during sprouting. Splitsstoesser et al. (25) and Andrews et al. (2) observed that aerobic plate counts of 6.0 and 4.0 log CFU/g, respectively, on mung bean seeds increased to ca. 8.0 log CFU/g within 2 days after germination. The population of mesophilic bacteria initially at ca. 7.0 log CFU/g of broccoli seed increased to ca. 8.0 and 9.0 log CFU/g within 3 and 5 days of sprouting, respectively (16).

Like TAB, numbers of MY were significantly higher on radish seeds (2.4 log CFU/g) than on alfalfa seeds (0.82 log CFU/g) and turnip seeds (1.69 log CFU/g). Andrews et al. (2) found that, of 750 mung bean seeds, 98% contained molds, including species capable of producing aflatoxin. MY were not detected on alfalfa and mung bean seeds, but ca. 4.0 log CFU/g were detected on sprouts 3 to 4 days after germination (8, 24). Significantly higher numbers of MY on sprouts, compared with those on seeds, may indicate that growth or introduction of MY from the environment occurred during sprouting or storing.

Salmonella, E. coli O157:H7, and E. sakazakii were not detected in radish, alfalfa, or turnip seeds. Gabriel (8) failed to isolate Salmonella from mung bean seeds but found that mung bean sprouts produced from these seeds were positive for Salmonella. It was assumed that seeds harbored Salmonella that were viable but not culturable.

This survey provides information on the microbiological quality of various types of vegetable sprouts and seeds.

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**Table 2. Populations of TAB and MY on radish, alfalfa, and turnip seeds**

<table>
<thead>
<tr>
<th>Type of seed</th>
<th>No. of samples</th>
<th>TAB (log CFU/g)</th>
<th>MY (log CFU/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Radish</td>
<td>33</td>
<td>4.08 A</td>
<td>2.42 A</td>
</tr>
<tr>
<td>Alfalfa</td>
<td>36</td>
<td>2.54 B</td>
<td>0.82 C</td>
</tr>
<tr>
<td>Turnip</td>
<td>27</td>
<td>2.84 B</td>
<td>1.69 B</td>
</tr>
</tbody>
</table>

*Within the same column, mean values that are followed by the same letter are not significantly different (P ≥ 0.05). The detection limit was 1 log CFU/g (10 CFU/g).*
commercially available to consumers in Korea. The high microbial contamination levels suggest a need to improve production practices used in the sprout industry in Korea. Further studies should focus on investigating methods to reduce microbial populations in seed handling as well as in sprout production and subsequent storage in the marketplace.

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