Research Paper

Microbial Antagonists of *Escherichia coli* O157:H7 on Fresh-Cut Lettuce and Spinach

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ABSTRACT

Fresh-cut lettuce and spinach can become contaminated with pathogens at numerous points from the field to the retail market. Natural microflora present on fresh produce may help reduce the pathogen load. The objective of this study was to isolate natural microflora from fresh-cut iceberg lettuce and baby spinach and to determine whether these bacteria were antagonistic toward *Escherichia coli* O157:H7. Samples were collected under conditions that mimicked actual practices between production and retail sale. Evidence of naturally occurring microorganisms on fresh lettuce (295 isolates) and spinach (200 isolates) and of possible antagonistic activity toward *E. coli* O157:H7 was documented. Inhibitory activity by several isolates was due to either acid production or antimicrobial peptides. Bacteria with inhibitory activity were isolated from every step in the processing and handling of the fresh-cut iceberg lettuce and baby spinach.

Although consumption of fresh produce is beneficial for optimal health, these foods may be associated with risks of foodborne illness. Reportable outbreaks of foodborne illness related to the consumption of fresh and minimally processed fruits and vegetables have increased dramatically since the 1970s (3, 7–9, 26). Contamination of fresh produce with human pathogens can occur at several points during growing, harvesting, processing, and handling (3–5, 15, 23, 24). Packers and processors currently use sanitizers in water that contacts fresh and fresh-cut produce, but a significant number of microorganisms usually remains on the product (1, 2, 21, 22). Because steps such as washing and sanitizing are not completely effective for removing microorganisms from produce, pathogens can remain on the product.

Produce commodities differ based on the site of harvest, postharvest handling procedures, and storage environment. Washing and storage routines also differ depending on the produce type (18). Thus, the typical microflora found on different types of produce from different areas can differ.

On many types of raw or minimally processed foods, the bacterial microflora often is composed of many species, and the activities of one bacterial species may influence the growth and activities of others. Competitive microorganisms can be found on fresh-cut produce (10, 12, 13, 16, 17, 20, 22, 25). The presence of these competitive microorganisms typically is determined at a particular stage in the handling of the product. Another approach would be to follow a product through typical processing and handling steps to determine whether the presence of these competitive microorganisms changes due to processing and handling.

Doering et al. (11) used the systems approach to evaluate the fate of *Escherichia coli* O157:H7 on fresh iceberg lettuce and spinach. The produce was inoculated with *E. coli* O157:H7 after harvesting, and contamination levels were determined at various stages of processing and handling from that point through storage in retail packages. On chopped iceberg lettuce and whole leaf spinach that was packaged and stored at 4°C, *E. coli* O157:H7 contamination could still be detected after typical handling practices, although populations decreased from initial levels in many cases by at least 1.5 log units. Samples collected as part of that study at the various stages of processing and handling were analyzed for the presence of competitive or antagonistic microorganisms. The objective of the present study was to identify microbial isolates that naturally occurred on fresh and packaged fresh-cut iceberg lettuce and baby spinach and were inhibitory to *E. coli* O157:H7 and to identify possible modes of this inhibition.

**MATERIALS AND METHODS**

**Experimental design.** This project was a companion study to that of Doering et al. (11). Doering et al. used the systems approach to determine the fate of *E. coli* O157:H7 in the presence of normal background microorganisms on iceberg lettuce and baby spinach under conditions that mimic actual practices used between production and retail sale. Lettuce and spinach inoculated with *E. coli* O157:H7 were processed and handled in ways that might occur in commercial situations (including variations in holding times before and after product cooling, transportation conditions and temperatures, washing treatments, and product storage temperatures and times). Aerobic mesophilic and psychrotrophic bacteria, coliforms (lettuce only), yeasts and molds, lactic acid bacteria, and *E. coli* O157:H7 populations were enumerated after each processing and handling step. Materials used and the pro-
FIGURE 1. Sampling points during simulated postharvest processing and handling of iceberg lettuce.

FIGURE 2. Sampling points during simulated postharvest processing and handling of spinach.

cessing, handling, and sampling procedures are detailed in their study (11). Figures 1 and 2 show the sampling points during the simulated postharvest processing and handling of iceberg lettuce and spinach.

At each sampling point, microbial isolates were randomly chosen from the various plating media used and were screened for inhibitory action against E. coli O157:H7. Isolates exhibiting inhibitory activity were characterized based on their morphological and biochemical properties and possible inhibitory activity.

Isolate selection. For each lettuce and spinach sample and media type, the Harrison disk method (14) was used to select 8 random colonies from plates containing less than 250 colonies. A cutout of the Harrison disk grid was used under the original plates to randomize colony selection.

Preparation of E. coli O157:H7. E. coli O157:H7 strains ATCC 43888 (human fecal isolate), EO122 (cattle isolate), K3995 (spinach isolate), and F4546 (alfalfa sprout outbreak isolate) (obtained from Li Ma and Larry Beuchat, Center for Food Safety, University of Georgia, Griffin) were used in this study to determine the inhibitory activity of the isolates selected from spinach and lettuce. Each strain was transferred into 9 ml of tryptic soy broth (Becton Dickinson, Sparks, MD) with 50 μg/ml ampicillin (Sigma-Aldrich, St. Louis, MO) (TSB+A) and incubated at 37°C in 24-h intervals. One milliliter of each strain was transferred into 9 ml of TSB+A for 3 days before the experiment. E. coli O157:H7 strains were centrifuged (Allegra X-22R centrifuge, Beckman Coulter, Fullerton, CA) at 4,000 g for 10 min. Each strain was then diluted to approximately 2 × 10^5 CFU/ml, and equal portions were combined to form an inoculum cocktail. The cocktail was spiral plated onto plate count agar (PCA; Becton Dickinson) plates using the lawn mode setting on a spiral plater (AutoPlate 4000, Spiral Biotech, Bethesda, MD), which plated approximately 5 × 10^3 CFU of the cocktail per 100 mm² of plate surface area (lawn plates). The lawn mode produced uniform microbial growth on the entire surface of the plate by depositing the inoculum at a constant rate.

Screening of isolates for inhibitory action. Inhibitory activity produced by the spinach and lettuce isolates was determined using the agar spot test as previously described by Fleming et al. (12). Each isolate selected was taken from the original TSA plate and placed onto a lawn plate of E. coli O157:H7 prepared that day. These plates were incubated at 37°C for 24 h and examined for zones of inhibition surrounding each isolate colony. Zone diameters were measured with an electronic caliper (Digimatic Slide, Scienceware, Pequannock, NJ).

Characterizing isolates. Cellular morphology and Gram reaction were determined for each isolate by microscopic examination of Gram stains of 20- to 24-h cultures. Identification of isolates was based on biochemical reactions. All isolates were tested for catalase and oxidase (14). The API 20NE test system (bioMérieux, Marcy l’Etoile, France) was used for nonenteric, oxidase-positive, gram-negative rods, and the API 20E test system (bioMérieux) was used for nonfastidious, gram-negative, oxidase-negative rods.

Acid production assay. Inhibition of the E. coli O157:H7 cocktail also was tested using the method of Lewus et al. (19). Isolates that exhibited inhibition on the E. coli O157:H7 cocktail lawn plates were cultured in 9 ml of TSB incubated at 37°C for 24 h. Serial dilutions were prepared for each sample so that 30 to 300 colonies would grow on each plate of PCA and tryptic soy agar without dextrose but with 0.5% yeast extract supplement (TSAw/o+YE; Becton Dickinson). These plates were incubated at 37°C for 24 h, and colony growth was checked for 30 to 300 colonies. After incubation, the colonies were overlaid with brain
heart infusion agar (BHI; Becton Dickinson) seeded with the same E. coli O157:H7 cocktail as used for the lawn plates at a population of 10^5 CFU/ml. PCA plates were used to allow acid production, and TSAw/o+YE plates were used to eliminate acid production due to the presence of dextrose. After solidification of the agar, the plates were incubated at 37°C for 24 h and examined for zones of inhibition.

**Protease sensitivity assay.** Sensitivity of the isolates’ metabolic by-products to proteases was examined to determine whether antimicrobial peptides were produced. The method described by Lewus et al. (19) was used. Isolates were grown in TSB at 37°C for 24 h. The proteases used in this experiment were protease type XXI (from Streptomyces griseus, 23 units per mg solid; Sigma-Aldrich), trypsin (from bovine pancreas, 1,000 units per mg solid; Sigma-Aldrich), α-chymotrypsin (from bovine pancreas, type II, 51 units per mg solid; Sigma-Aldrich), proteinase K (from Tritirachium album, 11.3 units per mg solid; Sigma-Aldrich), and pepsin (from porcine stomach mucosa, 3,460 units per mg solid; Sigma-Aldrich). These proteases were prepared immediately before use by adding 10 mg/ml sterile deionized water. Two microliters of each protease was spotted on to TSAYE (Becton Dickinson) plates and allowed to diffuse into the medium. Isolates to be tested were spot inoculated adjacent to the spotted proteases. Positive controls without protease and negative controls with sterile deionized water spotted in place of the proteases also were prepared. After 2 h to allow for diffusion and drying, plates were overlaid with 5 ml of BHI agar seeded with an E. coli O157:H7 cocktail to yield a final concentration of 10^5 CFU/ml on the plate. Plates were incubated for 24 h at 37°C and examined for the absence of zones of inhibition. Absence of the inhibition zone (as compared with the control isolate spot and the negative sterile water spots) indicated sensitivity to that particular protease and confirmed that the isolate produced an inhibitory protein.

**RESULTS**

Nine hundred forty-five lettuce samples were processed. From each of the different types of microbiological media used for each sample, colonies were isolated, resulting in 36,970 isolates that were screened for antagonistic activity toward E. coli O157:H7. Of these, 295 isolates had inhibitory activity against E. coli O157:H7. Of the 295 inhibitory lettuce isolates, 287 (97%) were gram-negative bacilli, 4 were gram-positive bacilli, and 3 were gram-positive cocci; 211 were oxidase negative, and 84 were oxidase positive. The gram-positive isolates were not identified further.

Four hundred sixty-two spinach samples were processed, yielding 17,513 isolates. When these isolates were tested for antagonistic activity toward E. coli O157:H7, 200 colonies had some inhibition. Of the 200 inhibitory spinach isolates, 197 (99%) were gram-negative bacilli, and 3 (1%) were gram-positive bacilli; 147 were oxidase negative, and 49 were oxidase positive. The gram-positive isolates were not identified to the genus level.

Of the 514 isolates, 356 (69%) were recovered from violet red bile agar, 84 (16%) were from psychrotrophic plates, 60 (12%) were from mesophilic plates, 7 (1%) were non-O157 isolates from TSA+A, 5 (1%) were from dichloran rose Bengal chlorotetacycline agar, and 2 (>1%) were from anaerobic Petrifilm. Three hundred sixty-six (71%) were from the 25°C field temperature samples, and 145 (28%) were recovered from the 32°C field temperature samples. Equal numbers of isolates were recovered from the 0, 10, and 72 h postcooling samples. Two hundred thirty-one (45%) were isolated from samples after washes, and 267 (52%) were isolated before chlorine washes. Tables 1 through 4 show the distribution of the isolates selected from the iceberg lettuce and spinach at the various processing and handling stages.

Among the bacterial isolates that exhibited inhibitory activity against E. coli O157:H7, the most common isolates obtained from multiple processing and storage steps were members of the genera Pantoea, Pseudomonas, Klebsiella, Enterobacter, Aeromonas, and Burkholderia. Serratia and Kluyvera isolates were slightly less common. At least 10 other genera were less frequently represented.

Inhibition due to the possible activity of antimicrobial peptides is noted in Tables 1 through 4. Although additional evaluation would be needed to identify these peptides, they might have been bacteriocins. For each test, inhibition was considered due to a possible antimicrobial peptide when no zone of inhibition was seen on the plate for any of the five proteases. Therefore, when one protease prevented inhibition, the test was scored as positive for antimicrobial peptide inhibition. Approximately 17% of the isolates produced an antimicrobial peptide as part of their inhibitory activity.

Acid production results also are noted in Tables 1 through 4. When the TSAYE and the PCA plates both indicated inhibition, the result was recorded as negative. When only the PCA plate indicated inhibition, the test was recorded as positive for acid inhibition. When both plates had growth and no evidence of inhibition, the test was recorded as negative. When only the TSA plate indicated inhibition, the test was considered inconclusive. Approximately 16% of the isolates produced acid as a part of their inhibitory activity.

**DISCUSSION**

Because of recent outbreaks of Salmonella and E. coli O157:H7 infections associated with fresh fruits and vegetables, it is becoming necessary to look at other options and multiple hurdles to minimize contamination. Fresh fruits and vegetables may retain much of their naturally occurring microflora even after handling and processing steps are complete. Pathogens may be introduced into this microflora and may or may not proliferate based on interactions with beneficial or nonpathogenic microorganisms present on the product.

This study was conducted to evaluate the natural microflora from multiple samples of fresh-cut lettuce and spinach under conditions that mimic those from the field to the finished packaged product. Four hundred ninety-five inhibitors of E. coli O157:H7 were isolated from fresh-cut lettuce and baby spinach. Naturally occurring microorganisms on foods have been recognized as having inhibitory activity against some foodborne pathogens. Bredholt et al. (6) found that lactic acid bacteria isolated from meat typically inhibit E. coli O157:H7 and Listeria monocytogenes. In other studies, fresh-cut produce has been a source of competitive microorganisms (11–13, 16, 20, 22, 25).
### TABLE 1. Distribution of microbial isolates that exhibited inhibitory activity against E. coli O157:H7; isolates were selected from iceberg lettuce initially held at 25°C prior to further processing

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Transport temp</th>
<th>Wash treatment</th>
<th>Storage temp of packaged lettuce</th>
<th>Days stored in retail package</th>
<th>Most common inhibitory isolates&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cooled, packaged immediately</td>
<td>4°C</td>
<td>12°C</td>
<td>Cl</td>
<td>No Cl</td>
<td>None</td>
</tr>
<tr>
<td>Cooled, 10 h post-cool</td>
<td>28</td>
<td>10</td>
<td>6</td>
<td>22</td>
<td>10</td>
</tr>
<tr>
<td>Cooled, 72 h post-cool</td>
<td>31</td>
<td>29</td>
<td>28</td>
<td>32</td>
<td>0</td>
</tr>
<tr>
<td>10 h precool, cooled, packaged immediately</td>
<td>10</td>
<td>10</td>
<td>11</td>
<td>9</td>
<td>0</td>
</tr>
<tr>
<td>10 h precool, cooled, 10 h post-cool</td>
<td>2</td>
<td>4</td>
<td>3</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>10 h precool, cooled, 72 h post-cool</td>
<td>2</td>
<td>17</td>
<td>9</td>
<td>6</td>
<td>3</td>
</tr>
</tbody>
</table>

<sup>a</sup> After coring, lettuce was either immediately cooled to 4°C or was held for 10 h before cooling (precool). Packaging of processed lettuce occurred either immediately or after holding for 10 or 72 h (postcool).

<sup>b</sup> Numbers in parentheses are the number of isolates of that genus that were most common for that treatment group and those exhibiting inhibitory activity due to antimicrobial peptides or acid production.

<sup>c</sup> At least one isolate from this genus produced detectable antimicrobial peptides as possible inhibitory substances.

<sup>d</sup> At least one isolate from this genus produced detectable acid as a possible inhibitory substance.

### TABLE 2. Distribution of microbial isolates that exhibited inhibitory activity against E. coli O157:H7; isolates were selected from iceberg lettuce initially held at 32°C prior to further processing

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Transport temp</th>
<th>Wash treatment</th>
<th>Storage temp of packaged lettuce</th>
<th>Days stored in retail package</th>
<th>Most common inhibitory isolates&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cooled, packaged immediately</td>
<td>4°C</td>
<td>12°C</td>
<td>Cl</td>
<td>No Cl</td>
<td>None</td>
</tr>
<tr>
<td>Cooled, 10 h post-cool</td>
<td>7</td>
<td>6</td>
<td>5</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>Cooled, 72 h post-cool</td>
<td>3</td>
<td>16</td>
<td>6</td>
<td>13</td>
<td>0</td>
</tr>
<tr>
<td>10 h precool, cooled, packaged immediately</td>
<td>12</td>
<td>3</td>
<td>2</td>
<td>13</td>
<td>0</td>
</tr>
<tr>
<td>10 h precool, cooled, 10 h post-cool</td>
<td>9</td>
<td>3</td>
<td>2</td>
<td>10</td>
<td>1</td>
</tr>
<tr>
<td>10 h precool, cooled, 72 h post-cool</td>
<td>9</td>
<td>11</td>
<td>8</td>
<td>12</td>
<td>0</td>
</tr>
</tbody>
</table>

<sup>a</sup> After coring, lettuce was either immediately cooled to 4°C or was held for 10 h before cooling (precool). Packaging of processed lettuce occurred either immediately or after holding for 10 or 72 h (postcool).

<sup>b</sup> Numbers in parentheses are the number of isolates of that genus that were most common for that treatment group and those exhibiting inhibitory activity due to antimicrobial peptides or acid production.

<sup>c</sup> At least one isolate from this genus produced detectable acid as a possible inhibitory substance.

<sup>d</sup> At least one isolate from this genus produced detectable antimicrobial peptides as possible inhibitory substances.
TABLE 3. Distribution of microbial isolates that exhibited inhibitory activity against E. coli O157:H7; isolates were selected from baby spinach initially held at 25°C prior to further processing

<table>
<thead>
<tr>
<th>Treatment groupa</th>
<th>Transport temp (4°C)</th>
<th>Wash treatment</th>
<th>Storage temp of packaged spinach</th>
<th>Days stored in retail package</th>
<th>Most common inhibitory isolatesb</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cl</td>
<td>No Cl</td>
<td>None</td>
<td>4°C</td>
<td>25°C</td>
</tr>
<tr>
<td>Cooled, packaged immediately</td>
<td>25</td>
<td>8</td>
<td>14</td>
<td>3</td>
<td>11</td>
</tr>
<tr>
<td>Cooled, 10 h postcool</td>
<td>25</td>
<td>17</td>
<td>8</td>
<td>0</td>
<td>14</td>
</tr>
<tr>
<td>Cooled, 72 h postcool</td>
<td>20</td>
<td>11</td>
<td>8</td>
<td>1</td>
<td>11</td>
</tr>
<tr>
<td>10 h precool, cooled, packaged immediately</td>
<td>20</td>
<td>12</td>
<td>6</td>
<td>2</td>
<td>16</td>
</tr>
<tr>
<td>10 h precool, cooled, 10 h postcool</td>
<td>31</td>
<td>22</td>
<td>9</td>
<td>0</td>
<td>18</td>
</tr>
<tr>
<td>10 h precool, cooled, 72 h postcool</td>
<td>30</td>
<td>11</td>
<td>15</td>
<td>4</td>
<td>21</td>
</tr>
</tbody>
</table>

a Spinach was either immediately cooled to 4°C or was held for 10 h before cooling (precool). Packaging of processed spinach occurred either immediately or after holding for 10 or 72 h (postcool).
b Numbers in parentheses are the number of isolates of that genus that were most common for that treatment group and those exhibiting inhibitory activity due to antimicrobial peptides or acid production.
c At least one isolate from this genus produced detectable acid as a possible inhibitory substance.
d At least one isolate from this genus produced detectable antimicrobial peptides as possible inhibitory substances.

TABLE 4. Distribution of microbial isolates that exhibited inhibitory activity against E. coli O157:H7; isolates were selected from baby spinach initially held at 32°C prior to further processing

<table>
<thead>
<tr>
<th>Treatment groupa</th>
<th>Transport temp (4°C)</th>
<th>Wash treatment</th>
<th>Storage temp of packaged spinach</th>
<th>Days stored in retail package</th>
<th>Most common inhibitory isolatesb</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cl</td>
<td>No Cl</td>
<td>None</td>
<td>4°C</td>
<td>25°C</td>
</tr>
<tr>
<td>Cooled, packaged immediately</td>
<td>17</td>
<td>8</td>
<td>7</td>
<td>2</td>
<td>8</td>
</tr>
<tr>
<td>Cooled, 10 h postcool</td>
<td>11</td>
<td>6</td>
<td>5</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>Cooled, 72 h postcool</td>
<td>8</td>
<td>7</td>
<td>1</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>10 h precool, cooled, packaged immediately</td>
<td>3</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>10 h precool, cooled, 10 h postcool</td>
<td>4</td>
<td>3</td>
<td>0</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>10 h precool, cooled, 72 h postcool</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>2</td>
</tr>
</tbody>
</table>

a Spinach was either immediately cooled to 4°C or was held for 10 h before cooling (precool). Packaging of processed spinach occurred either immediately or after holding for 10 or 72 h (postcool).
b Numbers in parentheses are the number of isolates of that genus that were most common for that treatment group and those exhibiting inhibitory activity due to antimicrobial peptides or acid production.
c At least one isolate from this genus produced detectable acid as a possible inhibitory substance.
d At least one isolate from this genus produced detectable antimicrobial peptides as possible inhibitory substances.
Liao and Fett (20) documented inhibitory action against *Salmonella enterica* serovar Chester, *L. monocytogenes*, and *E. coli* by *Pseudomonas* species on green pepper, romaine lettuce, baby carrots, alfalfa, and clover. The six isolates that inhibited at least one pathogen were *Bacillus* spp. (three isolates), *Pseudomonas aeruginosa* (one isolate), *Pseudomonas fluorescens* (one isolate), and a yeast (one isolate). On green pepper disks inoculated with the *P. fluorescens* and the yeast, growth of *Salmonella* Chester and *L. monocytogenes* was reduced by 1 and 2 log units, respectively, over a period of 3 days.

Schuenzel and Harrison (22) screened isolates from fresh-cut produce for antimicrobial activity against *Staphylococcus aureus*, *E. coli* O157:H7, *L. monocytogenes*, and *Salmonella enterica* serovar Montevideo. Of the 1,180 isolates screened, 37 (3.22%) had various degrees of inhibitory activity against at least one pathogen. Many isolates were inhibitory against all four pathogens. Bacteria that were most inhibitory were originally isolated from finished lettuce shreds.

Jablonske et al. (16) tested the interactions of *E. coli* O157:H7, *Salmonella Typhimurium*, and *L. monocytogenes* on plants cultivated in a gnotobiotic system. Only *Enterobacter cloacae* reduced *E. coli* O157:H7 and *L. monocytogenes* levels by approximately 1 log CFU/g on lettuce.

Cooley et al. (10) investigated *E. coli* O157:H7 survival and growth on lettuce in the presence of epiphytic bacteria. *E. coli* O157:H7 survival was enhanced 6-fold in the presence of the epiphyte *Wauseria paucula*, but *E. coli* O157:H7 survival was decreased 20- to 30-fold in the presence of *Enterobacter asburiae*. The researchers concluded that *E. asburiae* may outcompete *E. coli* O157:H7 by better utilization of some carbon and nitrogen substrates and suggested that the use of good agricultural practices would encourage the growth of competing bacteria.

This present study was not designed to determine all the modes of inhibition used by these isolates. The methods of Lewus et al. (19) helped to determine whether the mode of inhibition was due to the presence of antimicrobial peptides or acid production. In this study, the most effective inhibitory isolates were most prevalent on produce after the nonchlorine washings compared with after the chlorine washings, even after 18 days in product storage. Therefore, although the chlorine washings are beneficial for killing pathogenic microbes, the process may also reduce the numbers of the inhibitory nonpathogenic microbes on the produce. As found in other studies investigating antagonistic microflora on edible plant material, the majority of bacteria that exhibited inhibitory effects against *E. coli* O157:H7 were gram negative (10, 16, 20, 22). *Pantoea* was resilient, was recovered at every processing step, and had the greatest degree of inhibition for most samples. None of the handling or processing steps appeared to decrease the amount or inhibitory activity of these natural isolates. Even after 18 days of storage, *Pantoea* isolates had inhibition capabilities similar to those of isolates recovered during the earlier processing and handling stages.

The presence on fresh lettuce and spinach of a variety of naturally occurring microorganisms with possible antagonistic activity toward *E. coli* O157:H7 was documented in this study. Those microorganisms that pose no potential health threat may prove beneficial. However, several hurdles must be overcome before antagonistic microorganisms can be used in a biocontrol strategy with fresh or fresh-cut produce. The safety of the inhibitory microorganism would be of foremost concern, but practical application methods also must be developed. For example, it would be impractical to inoculate large numbers of competitive bacteria onto the product, especially if the microorganism of interest were a potential spoilage agent. Any benefit gained from the inhibition of a foodborne pathogen would be lost if the product spoiled more rapidly. This study was a companion study to that conducted by Doering et al. (11). In that study, the *E. coli* O157:H7 populations on iceberg lettuce and spinach stored in retail packages decreased over time by up to 1.5 log units. Native microflora with inhibitory activity against *E. coli* O157:H7 may be useful in the fight against foodborne pathogens.

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**REFERENCES**


