Short communication

Presence of indicator bacteria, *Salmonella* and diarrheagenic *Escherichia coli* pathotypes on mung bean sprouts from public markets in Pachuca, Mexico

Jorge F. Cerna-Cortes a, Carlos A. Gómez-Aldapa b, Esmeralda Rangel-Vargas b, Enrique Ramírez-Cruz b, Javier Castro-Rosas b, *a*

a Departamento de Microbiología, Escuela Nacional de Ciencias Biológicas-IPN, Prolongación Carpio y Plan de Ayala s/n, Col. Casco de Santo Tomas, Del. Miguel Hidalgo, 11340 Mexico, D.F., Mexico

b Centro de Investigaciones Químicas, Instituto de Ciencias Básicas e Ingeniería, Universidad Autónoma del Estado de Hidalgo, Centro Universitario, Carretera Pachuca-Tulancingo Km. 4.5, 42383 Mineral de la Reforma, Hidalgo, Mexico

A B S T R A C T

Coliform bacteria (CB), fecal coliforms (FC), *Escherichia coli*, diarrheagenic *E. coli* pathotypes (DEP) and *Salmonella* frequencies were determined for mung bean (*Vigna radiata*) sprouts. One hundred sprout samples were collected from markets in Pachuca, Hidalgo state, Mexico. Of these samples, 100% were positive for CB, 98% for FC, 95% for *E. coli*, 10% for DEP and 5% for *Salmonella*. Identified DEP included enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC) and Shiga toxin-producing *E. coli* (STEC). The ETEC and EIEC were each isolated from 2% of samples, and the STEC from 6% of samples. No *E. coli* O157:H7 were detected in any STEC-positive samples. Positive correlations were observed between FC and *E. coli* and between *E. coli* and DEP. A negative correlation occurred between CB and DEP, and between CB and *Salmonella*. Neither FC nor *E. coli* correlated with *Salmonella* presence in the sprout samples. This is the first report of ETEC, EIEC and STEC isolated from sprouts in Mexico and the first report of *Salmonella* isolation from mung bean in Mexico. Mung bean sprouts are very probably an important factor contributing to the endemicity of ETEC, EIEC and STEC and *Salmonella*-related gastroenteritis in Mexico.

© 2012 Elsevier Ltd. All rights reserved.

1. Introduction

During the last decade, consumption of vegetable seed sprouts has increased in many countries because they are relatively easy to produce and perceived as having high nutritional value. Greater consumption has led to a concurrent rise in the number of sprout-associated foodborne illness outbreaks, with at least 40 outbreaks reported in several countries (Health Canada, 2012). Alfalfa and mung bean sprouts are most often implicated in outbreaks in North America (CDC, 2012; Fett, 2005; Health Canada, 2012), and the most frequently associated causative agents are *Salmonella* and *Escherichia coli* O157:H7 (CDC, 2012; Fett, 2005). Mung bean sprouts were responsible for a salmonellosis outbreak in Ontario, Canada, in 2005 which resulted in over 600 reported cases (Health Canada, 2012). Clearly alfalfa and mung bean sprouts constitute a significant food safety risk.

*Salmonella* and *S. Typhi* infections are endemic in Mexico; between 2009 and 2011, 381,320 salmonellosis cases and 139,000 typhoid fever cases were reported (Secretaria de Salud, 2012). In Mexico, *Salmonella* has been isolated from raw alfalfa sprouts (Castro-Rosas & Escartín, 1999), but there are no reports of *Salmonella* isolation from other seed sprouts such as mung bean.

Diarrheagenic *E. coli* pathotypes (DEP) are important foodborne pathogens (Kaper, Nataro, & Mobley, 2004). They are classified according to their virulence traits: enterotoxigenic *E. coli* (ETEC); enteropathogenic *E. coli* (EPEC); enteroinvasive *E. coli* (EIEC); enteroaggregative *E. coli* (EAEC); diffuse adherent *E. coli* (DAEC); Shiga toxin-producing *E. coli* (STEC) and enterogaugregative-haemorrhagic *E. coli* (EAHEC). Recent DEP outbreaks have been associated with a wider variety of vegetables. A recent foodborne outbreak involving EAHEC originating from sprouts in several European countries highlights the importance of screening for DEP in fresh vegetables and sprouts (Buchholz et al., 2011; Gault et al., 2011). Clearly, more extensive frequency of incidence data for pathogenic bacteria such as DEP is still needed for a wide variety of vegetables consumed raw. Health authorities in Mexico do not routinely test for DEP in foods or from people with acute diarrhea.

*Corresponding author. Tel.: +52 771 717 2000x6501; fax: +52 771 717 2000x6502.
E-mail address: jcastro@uaeh.edu.mx (J. Castro-Rosas).*
However, identification of DEP presence in food in Mexico is vital because they have been associated with diarrheal illness in both native children (Estrada-García et al., 2005, 2009; Paniagua et al., 2007) and visitors to the country (Merson et al., 1976; Paredes-Paredes et al., 2011).

Human pathogens can contaminate fresh vegetables as primary contamination (while growing and during harvest) or secondary contamination (during washing, slicing, soaking, packaging and preparation) (Harris et al., 2003; Sivapalasingam, Friedman, Cohen, & Tauxe, 2004). However, sprout-associated outbreaks have largely been linked to seeds contaminated with pathogenic microorganisms (NACMCF, 1999a), rather than post-production contamination.

In Mexico, sprouts are often eaten raw; this increases the potential infection risk associated with sprout consumption. No reported data exist on DEP frequency in any sprout types in Mexico, and neither are there data on Salmonella presence on mung bean sprouts from Mexico. The present study objective was to measure the presence and correlation of CB, FC, E. coli, DEP and Salmonella on mung bean sprouts.

2. Materials and methods

2.1. Raw material

There are 13 public markets in the city of Pachuca, Hidalgo state, Mexico, but only 4 are sizable. A total of 100 mung bean (Vigna radiata) sprout samples (150 g each) were purchased in the four largest public markets in Pachuca, immediately placed in a sterilized plastic bag, transported to the laboratory and processed within 1 h. At the moment of purchase, the sprouts (approximately 250 g) were packaged in plastic containers stored at room temperature.

2.2. Microbiological analyses

Subsamples (100 g) from each sample were placed in a sterile plastic bag and lactose broth (LB) added to reach a 1:10 (10⁻¹) final dilution. These subsamples were pumped into a stomacher for 1 min, and sample dilutions prepared for microorganism counts using peptone diluent (0.1%). Samples were analyzed to detect the presence of coliform bacteria (CB), fecal coliforms (FC) and E. coli using both the somatic polyvalent (O) test and flager- (H) test. Antiserum O and H were purchased from Antistore, France. Salmonella (Serotype Typhi, salmonella enterica), and Shiga toxin-producing E. coli (STEC) were isolated from 2% of the samples while the STEC were isolated from 6%. In positive samples, concentration ranged from 3.6 to 106 CFU/g reported by him. E. coli O157:H7 was detected in any STEC-loci detected positive samples (Table 2). For the STEC, the stxl locus was detected in four strains and both stxl and stx2 in two strains (Table 2).

2.3. Multiplex PCRs for DEP loci identification

A total of 280 E. coli strains isolated from the sprout samples were analyzed using two multiplex PCRs to identify several diarrheic E. coli (DE) loci, as described elsewhere (Cerna, Nataro, & Estrada-García, 2003; López-Saucedo et al., 2003). The first multiplex identifies the following loci: heat-stable and heat-labile enterotoxins (st, lt) for ETEC, intimin (eaeA) and bundle-forming pilus (bfp) for EPEC, Shiga toxin 1 and 2 (stx1, stx2) and intimin (eaeA) for STEC, and invasion-associated loci (lal) for EIEC. The second multiplex PCR identifies 3 EAEC plasmid-borne virulence genes including the master regulator (aggR), dispersin (aap) and the autotransporter Tol C (aatA). All E. coli strains analyzed by PCR assays were typical E. coli cultures (i.e. lactose positive, gas positive from lactose fermentation, gas positive from glucose fermentation, indole positive, methyl red positive, Voges proskauer negative, Citrate negative and lysine deoxycholate positive) positive. We have used these multiplex PCR procedures previously in a similar study (Castro-Rosas et al., 2012). STEC isolates were further characterized by expression of the O157 lipopolysaccharide and H7 flagellar antigens using only the E. coli O157:H7 latex agglutination test kit (RIMÈ E. coli O157:H7 Latex Test Kit, Remel, Lenexa, KS, USA).

2.4. Statistical analysis

The Pearson correlation coefficient was used to compare relationships between presence of CB, FC, E. coli, DEP and Salmonella. A p-value < 0.05 was considered significant. All statistical analyses were run with the Statistical program (StatSoft, Inc., Tulsa, version 8).

3. Results and discussion

The analyzed sprout samples had generally poor microbiological quality, with CB detected in 100% of samples, FC in 98%, E. coli in 95%, DEP in 10% and Salmonella in 5% (Table 1). Concentration of CB ranged from 3.2 up to 5.9 log CFU/g, whereas FC and E. coli concentrations were between 10⁴ and 10⁵ MPN/g. Identified DEP included enterotoxigenic E. coli (ETEC), enteroinvasive E. coli (EIEC) and Shiga toxin-producing E. coli (STEC). Both the ETEC and EIEC were isolated from 2% of the samples while the STEC were isolated from 6%. In positive samples, concentration ranged from 3.6 to 120 MPN/g for ETEC, 7.4 to 240 MPN/g for EIEC, and 3.6 to 1100 MPN/g for STEC. No E. coli O157:H7 were detected in any STEC-positive samples (Table 2). For the STEC, the stxl locus was detected in four strains and both stxl and stx2 in two strains (Table 2).

OB-CB levels coincide with the 5.5 × 10⁶ to 2.3 × 10⁷ CFU/g of raw soybean sprouts (average = 4.8 × 10⁶ CFU/g) reported previously (Kim, Lee, & Paik, 2004). In a study done in Norway, FC were isolated from 24% (n = 64) of mung bean sprout samples at

<table>
<thead>
<tr>
<th>Microorganism or indicator</th>
<th>Minimum</th>
<th>Median</th>
<th>Maximum</th>
<th>Frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CB</td>
<td>3.2</td>
<td>4.5</td>
<td>5.9</td>
<td>100</td>
</tr>
<tr>
<td>FC</td>
<td>&lt;3</td>
<td>43</td>
<td>1100</td>
<td>98</td>
</tr>
<tr>
<td>E. coli</td>
<td>&lt;3</td>
<td>11</td>
<td>1100</td>
<td>95</td>
</tr>
<tr>
<td>DEP</td>
<td>&lt;3</td>
<td>&lt;3</td>
<td>1100</td>
<td>10</td>
</tr>
<tr>
<td>Salmonella</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>5</td>
</tr>
</tbody>
</table>

ND = not determined.

* n = 100. Minimum, median and maximum values are in log₁₀ CFU per g for coliform bacteria and in most probable number (MPN) per g for fecal coliforms and E. coli.
Table 2
Identified diarrheagenic E. coli pathotypes (DEP) and loci, and DEP concentration on mung bean sprout samples.

<table>
<thead>
<tr>
<th>Sample</th>
<th>DEP</th>
<th>Loci</th>
<th>Concentration (MPN/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>ETEC</td>
<td>st</td>
<td>120</td>
</tr>
<tr>
<td>7</td>
<td>ETEC</td>
<td>st</td>
<td>3.6</td>
</tr>
<tr>
<td>16</td>
<td>Non-O157 STEC</td>
<td>stx1</td>
<td>15</td>
</tr>
<tr>
<td>33</td>
<td>Non-O157 STEC</td>
<td>stx1, stx2</td>
<td>1100</td>
</tr>
<tr>
<td>35</td>
<td>Non-O157 STEC</td>
<td>stx1</td>
<td>3.6</td>
</tr>
<tr>
<td>46</td>
<td>Non-O157 STEC</td>
<td>stx1</td>
<td>460</td>
</tr>
<tr>
<td>74</td>
<td>Non-O157 STEC</td>
<td>stx1</td>
<td>28</td>
</tr>
<tr>
<td>79</td>
<td>EIEC</td>
<td>iai</td>
<td>240</td>
</tr>
<tr>
<td>87</td>
<td>EIEC</td>
<td>iai</td>
<td>7.4</td>
</tr>
<tr>
<td>97</td>
<td>Non-O157 STEC</td>
<td>stx1, stx2</td>
<td>3.6</td>
</tr>
</tbody>
</table>

Concentrations ranging from $2 \times 10^3$ to $1.4 \times 10^7$ CFU/g sprouts (Robertson, Johannessen, Gjerde, & Loncarevic, 2002). Enterobacter spp. and Klebsiella spp. were isolated from most of the FC-negative samples in that study, and typically identified strains included Enterobacter cloacae, Cronobacter sakazakii and Klebsiella pneumoniae. E. coli was isolated from eight of the 62 FC-positive samples (Robertson et al., 2002).

Salmonella and E. coli O157:H7 are major foodborne disease-causing pathogens associated with sprout consumption. With the exception of E. coli O157:H7, there are no reports in the relevant literature on the frequency of other DEP in sprouted seeds intended for human consumption. In the present study, Salmonella was detected in 5% of the mung bean samples and DEP (ETEC, EIEC or STEC) in 10% (Table 1). This contrasts with the results of Robertson et al. (2002), who detected no Salmonella in alfalfa, mung bean, radish or mixed sprouts from Norway. In contrast, Salmonella has been reported in 94% of mung bean sprouts tested in the Philippines (Gabriel et al., 2007). In a study done in India, 23% of mung bean sprouts harbored Salmonella, although E. coli O157:H7 was not isolated from the samples (Saroj et al., 2007).

The microbiological contamination observed in the mung bean sprouts analyzed here may have been caused by the use of contaminated seeds (Mahon et al., 1997; NACMCF, 1999a). Contaminated seed is probably the source of most, if not all, sprout-associated disease outbreaks, since seed is frequently grown, milled and stored in conditions where contamination can occur readily (NACMCF, 1999a). Pathogenic bacteria contamination levels on seeds may be low, but sprouting conditions and processes are ideal for increasing Salmonella and E. coli counts, and for spreading a pathogenic agent throughout an entire production lot. Possible seed contamination routes are myriad (NACMCF, 1999a), but the primary cause is apparently treatment of seeds as a raw agricultural product rather than as a food product which may harbor microorganisms from its source environment. Indeed, most seed is used for agricultural purposes, and the decision to use it for sprouting is often not made until post-harvest (Robertson et al., 2002). Like any other vegetable intended for raw consumption, mung bean sprouts are a potential pathogen vehicle. Field sources of seed contamination with pathogenic microorganisms include soil, water, wild and domestic animals, drift and runoff from adjacent farms and manure (Beuchat, 1996; Natvig, Ingham, Ingham, Cooperband, & Roper, 2002). Some of these sources may not directly affect seeds sprouted for human consumption since they are normally grown in trays rather than in soil. Seeds also have cracks and cavities where presumptive pathogenic bacteria can survive and eventually create biofilms (Fett, 2000). In addition, multiple pathogenic microorganisms contamination sources occur during mung bean sprout packaging, distribution and sale.

In Mexico, DEP have been associated with cases of acute diarrhea in children which required hospitalization (Estrada-Garcia et al., 2005) and with community-acquired diarrhea (Estrada-Garcia et al., 2009). Non-O157 (STEC) strains have been identified in other food items in Mexico, and DEP have been isolated most frequently from food and beverages sold on the street in Mexico City (Cerna-Cortes, Vega-Negrete, Ortega-Villegas, Zaidi, & Estrada-Garcia, 2012; López-Saucedo, Cerna, & Estrada-Garcia, 2010), Enterotoxigenic E. coli (ETEC) (Estrada-Garcia, Cerna, Thompson, & López-Saucedo, 2002) and EIEC (López-Saucedo et al., 2003) strains have also been identified in chili sauces sold on the street in Mexico City. No O157 and H7 antigens were detected in any STEC-positive chili samples. These results coincide with previous reports on the presence of E. coli O157:H7 in Mexico (Callaway et al., 2004) and the absence of O157 and H7 antigens in STEC strains isolated in Mexico (Estrada-Garcia et al., 2009; López-Saucedo et al., 2003).

Positive correlations were observed in the present results between FC and E. coli, and between E. coli and DEP. However, no correlation existed between FC and Salmonella, and between E. coli and Salmonella. In addition, a negative correlation existed between CB and FC, E. coli, DEP and Salmonella (Table 3).

For many years, FC and E. coli have been used as indicators of fecal contamination of food. Numerous studies in the microbiology literature correlate fecal coliform levels with E. coli presence. However, the value of the fecal coliform assay as a fecal contamination indicator is nullified when non-fecal organism bacteria are the principal microbes detected by the assay. Indeed, FC are not effective fecal indicators in fresh vegetables (Nguyen-the & Carlin, 2000), partially because common non-fecal coliforms such as Klebsiella and Enterobacter can grow under thermotolerant test conditions (NACMCF, 1999b). Considered the best indicator of fecal contamination in foods, E. coli is probably the most commonly used indicator bacteria. Microbiological criteria have been regularly established for E. coli, but disagreement still exists as to whether its presence is necessarily associated with the presence of fecal matter or pathogenic bacteria.

Salmonella was isolated in the absence of E. coli in two samples. This coincides with reports of enteropathogenic microorganisms such as Salmonella being isolated from foods with no evidence of E. coli. Orozco, Rico-Romero, and Escartín (2008) isolated Salmonella from tomatoes free of E. coli, and Endley, Lu, Vega, Hume, and Pillai (2003) found no E. coli on three Salmonella-positive carrot samples from a total of 75 samples. After collection of 345 samples of fresh vegetables, Garcia-Villanova Ruiz, Vargas, and Garcia-Villanova (1987) found eight to be Salmonella-positive but E. coli-negative. Finally, Sago, Little, Ward, Gillespie, and Mitchell (2003) identified no E. coli on three out of five Salmonella-positive ready-to-eat salad samples (3852 samples). As a result, many researchers agree on the need to eliminate FC as an indicator group of the possible presence of pathogenic microorganisms, and on the increasing urgency of expanding the search for pathogenic microorganisms beyond just E. coli to include species such as Salmonella.

Table 3
Correlations between different indicator groups versus DEP and Salmonella.

<table>
<thead>
<tr>
<th>Variable pairs</th>
<th>Pearson r</th>
<th>$r^2$</th>
<th>p-Level*</th>
</tr>
</thead>
<tbody>
<tr>
<td>CB &amp; FC</td>
<td>0.12348</td>
<td>0.014969</td>
<td>0.225260</td>
</tr>
<tr>
<td>CB &amp; E. coli</td>
<td>-0.050413</td>
<td>0.002541</td>
<td>0.618407</td>
</tr>
<tr>
<td>CB &amp; DEP</td>
<td>-0.029320</td>
<td>0.000859</td>
<td>0.772273</td>
</tr>
<tr>
<td>CB &amp; Salmonella</td>
<td>0.028484</td>
<td>0.000834</td>
<td>0.775438</td>
</tr>
<tr>
<td>FC &amp; E. coli</td>
<td>0.277664</td>
<td>0.077097</td>
<td>0.005159</td>
</tr>
<tr>
<td>FC &amp; DEP</td>
<td>0.248117</td>
<td>0.061562</td>
<td>0.0029302</td>
</tr>
<tr>
<td>FC &amp; Salmonella</td>
<td>0.150059</td>
<td>0.022518</td>
<td>0.136179</td>
</tr>
<tr>
<td>E. coli &amp; DEP</td>
<td>0.880485</td>
<td>0.775254</td>
<td>0.000000</td>
</tr>
<tr>
<td>E. coli &amp; Salmonella</td>
<td>-0.063791</td>
<td>0.004069</td>
<td>0.528345</td>
</tr>
<tr>
<td>DEP &amp; Salmonella</td>
<td>-0.027160</td>
<td>0.000738</td>
<td>0.788521</td>
</tr>
</tbody>
</table>

*p-Level < 0.05 indicates a positive correlation.
The analyses in the present study focused on determining the microbiological quality of commercially available mung bean sprouts in a city in central Mexico. The high microbial contamination levels and presence of ETEC, EIEC and STEC and Salmonella found here highlight the need for improving practices in Mexico’s sprout producers. A number of measures can be taken to mitigate this risk in Mexico, including tighter regulation of production, shipping and marketing of mung bean and other sprouts, and informing the public about adequate handling of fresh store-bought products and proper hygiene practices when growing sprouts from seeds at home.

Although the number of mung bean sprouts samples analyzed here could be considered as limited, it was sufficient to conclude that DEP and Salmonella incidences on mung bean sprouts represent a potential public health risk. In fact, it is quite possible that increasing intake of sprouts from sources such as mung bean could be a significant factor contributing to the endemcity of ETEC, EIEC and STEC and Salmonella-related gastroenteritis in Mexico.

Acknowledgments

This research was funded by Fondos Mixtos de Fomento a la Investigación Científica y Tecnológica, Consejo Nacional de Ciencia y Tecnología – Gobierno del Estado de Hidalgo, Mexico (Grant No. 96887).

References


