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Survival of *Enterobacter sakazakii* on fresh produce as affected by temperature, and effectiveness of sanitizers for its elimination

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Abstract

A study was done to determine the survival characteristics of *Enterobacter sakazakii* on the surface of apples, cantaloupes, strawberries, lettuce, and tomatoes stored at 4, 12, and 25 °C for 8–28 days. Populations significantly decreased ($p \leq 0.05$) on all test produce at all storage temperatures. The efficacy of chlorine, chlorine dioxide, and a peroxyacetic acid-based sanitizer (Tsunami 200®) treatments (1 and 5 min) in killing the bacterium on apples, tomatoes, and lettuce was determined. Chlorine and chlorine dioxide, at ≥ 50 μg/ml, were equivalent in killing *E. sakazakii* on apples. Populations of *E. sakazakii* on apples treated with 10 μg/ml chlorine dioxide for 1 or 5 min were significantly reduced ($p \leq 0.05$) by 3.38 and 3.77 log CFU/apple, respectively, compared to the number remaining on apples after washing with water. Treatment with Tsunami 200 at 40 μg/ml for 1 min caused reductions of ≥ 4.00 log CFU/apple. Reductions of ≥ 3.70 log CFU/tomato were achieved by treatment with 10 μg/ml chlorine or chlorine dioxide or 40 μg/ml Tsunami 200 for 5 min. Reductions in populations of *E. sakazakii* on lettuce treated with chlorine at 10, 50, and 100 μg/ml for 1 min ranged from 1.61 to 2.50 log CFU/sample (26 ± 4 g), compared to populations remaining on lettuce washed with water. Chlorine was less effective in killing *E. sakazakii* on lettuce than on apples or tomatoes. Treatment of lettuce with Tsunami 200 (40 and 80 μg/ml) for 5 min caused a reduction of ≥ 5.31 log CFU/sample. Results provide insights to predicting survival characteristics of *E. sakazakii* on produce and the efficacy of sanitizers in killing the bacterium.

Keywords: *Enterobacter sakazakii*; Fruit; Vegetable; Apple; Cantaloupe; Strawberry; Lettuce; Tomato; Chlorine; Chlorine dioxide; Peroxyacetic acid

1. Introduction

During the past two decades, consumption of fresh fruits and vegetables has increased in the U. S. Concomitant with this trend, the number and frequency of outbreaks of illness associated with fresh produce have increased (Harris et al., 2003). Outbreaks of *Escherichia coli* O157:H7 infections, for example, have been linked to lettuce (Hilborn et al., 1999) and alfalfa sprouts (Breuer et al., 2001) and salmonellosis has been associated with consumption of tomatoes (Cummings et al., 2001) and cantaloupe (Centers for Disease Control and Prevention, 2002). Factors affecting survival and growth characteristics of foodborne pathogens on fresh and fresh-cut produce that may impact the level of risk of causing infections have not been fully defined.

Enterobacter sakazakii is an emerging foodborne pathogen known to cause meningitis (Burdette and Santos, 2000; Gallagher and Ball, 1991), sepsis (Simmons et al., 1980), bacteremia (Noriega et al., 1990), and necrotizing enterocolitis (Van Acker et al., 2001) in preterm neonates and immunocompromised adults (Lai, 2001). This bacterium has been found in several types of foods, food processing plants, and the environment (Iversen and Forsythe, 2003; Kandhai et al., 2004; Gurtler et al., 2005), although outbreaks of infection have been associated primarily with reconstituted, infant formula (Himelright et al., 2002; Muytjens and Kolee, 1990; Van Acker et al., 2001). While *E. sakazakii* has not been reported to cause illnesses linked to the consumption of fresh produce, it has been isolated from lettuce (Soriano et al., 2001) and other vegetables

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(Leclercq et al., 2002), thereby representing a potential risk of causing human infections. We have observed that *E. sakazakii* can grow on several types of fresh-cut produce and in fruit and vegetable juices (Kim and Beuchat, 2005).

Chlorinated water, chlorine dioxide (gaseous and aqueous), and peracetic acid-based sanitizers are among the chemical treatments used to reduce populations of microorganisms on fresh fruits and vegetables. Water containing free chlorine concentrations of 50–200 μg/ml can be used to sanitize produce (Beuchat, 1998; U.S. Food and Drug Administration, 2001). Chlorine has been shown to be effective in reducing populations of *E. coli* O157:H7 (Beuchat, 1999; Park and Beuchat, 1999; Fett, 2002a; Ryu and Beuchat, 2005), *Salmonella* (Zhuang et al., 1995; Park and Beuchat, 1999; Weissinger et al., 2000; Fett, 2002a), and *Listeria monocytogenes* (Ukuku and Fett, 2002; Beuchat et al., 2004) on fresh produce. Aqueous chlorine dioxide has been reported to kill *E. coli* O157:H7 on lettuce and baby carrots (Singh et al., 2002) and *E. coli* O157:H7 and *L. monocytogenes* on green peppers (Han et al., 2001), apples, lettuce, strawberries, and cantaloupes (Rodgers et al., 2004). Peroxyacetic acid has been used to reduce microbial populations in process water (Hilgren and Salverda, 2000) and on apples (Wisniewsky et al., 2000; Rodgers et al., 2004), cantaloupes (Park and Beuchat, 1999; Rodgers et al., 2004), lettuce (Beuchat et al., 2004; Rodgers et al., 2004), strawberries (Rodgers et al., 2004), honeydew melons, and asparagus (Park and Beuchat, 1999). However, the efficacy of sanitizers in killing *E. sakazakii* on fresh fruits and vegetables has not been reported.

An objective of this study was to determine the survival characteristics of *E. sakazakii* on the surface of apples, cantaloupes, strawberries, lettuce, and tomatoes stored at 4, 12, and 25 °C for up to 28 days. A second objective was to determine the effectiveness of chlorine, aqueous chlorine dioxide, and a peroxyacetic acid-based sanitizer in killing *E. sakazakii* inoculated in an organic carrier (horse serum) onto the surface of apples, tomatoes, and lettuce.

2. Materials and methods

2.1. Preparation of inoculum

Five clinical isolates of *E. sakazakii* (strain 4923, A9002, 1625, 8397, and LCDC 674) were grown in tryptic soy broth (TSB; BBL/Difco, Sparks, Maryland) at 37 °C for 24 h. Nalidixic acid-adapted cells of each strain were selected by surface plating 0.1 ml of a 24-h culture on tryptic soy agar (TSA; BBL/Difco) supplemented with nalidixic acid (50 μg/l) and pyruvate (0.1%) (TSANP) and incubating plates at 37 °C for 24 h. Nalidixic acid-adapted cells were picked from colonies and cultured in TSB supplemented with 1% glucose and nalidixic acid (50 μg/l) (TSBGN) at 37 °C for 24 h. After three consecutive transfers of ca. 10 μl into TSBGN at 24-h intervals, cells from 30 ml of culture of each strain were collected by centrifugation (1250 ×*g*, 15 min, 21 °C) and the supernate was decanted. Cells were resuspended in 15 ml of sterile 5% horse serum (Sigma-Aldrich, St. Louis, Missouri) and appropriate volumes of suspensions of each strain were combined to give approximately equal populations (10 log CFU/ml) of each strain.

2.2. Preparation of fresh produce

Unwaxed organically grown apples (*Malus domestica* Borkh., Red Delicious cv.) and unwaxed organically grown tomatoes (*Lycopersicon esculentum* Mill, Roma cv.) were purchased at a local farmers' market 2–3 days prior to use in experiments. Cantaloupes (*Cucumis melo* var. *cantalupensis*), strawberries (*Fragaria virginiana* Duschesne), and iceberg lettuce (*Lactuca sativa* L.) were purchased at a retail grocery store 1 day prior to use in experiments. After purchase, apples, tomatoes, and cantaloupes were stored at room temperature (22±2 °C) and lettuce and strawberries were kept at 12 °C until inoculated. Two or three of the wrapper leaves were removed from each head of lettuce and discarded. Samples of lettuce subjected to inoculation and analysis consisted of three 9×9 cm pieces cut from the 2–3 leaves beneath the wrapper leaves. Two 5×5 cm areas marked on the rind surface of each cantaloupe (1150±70 g) served as inoculation sites from which samples were eventually excised and analyzed. Each sample of apple (186±24 g) and tomato (82±9 g) consisted of a single fruit. A sample of lettuce consisted of three 9×9 cm pieces (26±4 g) and a sample of strawberries consisted of three fruits (50±4 g total).

2.3. Storage test

2.3.1. Inoculation and storage of produce

Each produce sample was placed on a wire screen elevated 7 cm above the work surface in a laminar flow biosafety cabinet. Using a micropipette, 100 μl of the five-strain mixture of *E. sakazakii* was deposited on the surface of each produce to give ca. 9 log CFU/produce sample. The inoculated produce was dried for 2 h (35% relative humidity) at 22±2 °C in the laminar flow hood. Inoculated apples, cantaloupes, strawberries, and tomatoes were placed in separate plastic trays and lettuce samples were placed in stomacher 400 bags (Seward Medical, Ltd., London, UK). Each type of inoculated produce was placed in a separate plastic tub and stored at 4, 12, or 25 °C for up to 28 days before being subjected to microbiological analysis; respective atmospheric relative humidities at the three storage temperatures were 85, 45, and 45%.

2.3.2. Microbiological analysis

Populations of *E. sakazakii* on inoculated produce stored at 4, 12, and 25 °C for 0 (2 h after inoculation), 1, 4, 7, 8, 14, 21, and/or 28 days were determined. At each sampling time, apple, tomato, and strawberry samples were transferred to quart-sized (0.95 l) Ziploc® bags (S. C. Johnson, Racine, Wisconsin). Fifty milliliters of sterile 0.1% peptone water were added to each bag. Apples and tomatoes were firmly hand-rubbed and strawberries were gently shaken for 1 min. Tissues from inoculated areas (5×5 cm, 0.5 cm deep) on the rind of inoculated cantaloupes were removed using a sterile stainless steel scalpel and placed in stomacher 400 bags containing a filter septum. Fifty milliliters of 0.1% peptone water were added to each bag containing a