# *ENTEROBACTER SAKAZAKII*

## PERFORMANCE OF MEDIA FOR RECOVERING STRESSED CELLS OF *ENTEROBACTER SAKAZAKII* AS DETERMINED USING SPIRAL PLATING AND ECOMETRIC TECHNIQUES

(J. B. Gurtler and L. R. Beuchat)

The method used by the U.S. Food and Drug Administration to detect *Enterobacter sakazakii* in powdered infant formula requires rehydration in sterile distilled water overnight at 36°C, followed by enrichment in Enterobacteriaceae enrichment (EE) broth overnight at 36°C, surface plating and streaking on violet red bile glucose (VRBG) agar, incubating overnight at 36°C, subculturing presumptive-positive colonies in tryptic soy agar (TSA), and incubating plates for 48 - 72 h at 36°C. Yellow-pigmented presumptive positive *E. sakazakii* colonies are then subjected to confirmation tests using the API 20E biochemical identification system, which requires an additional 18 - 24 h. EE broth and VFBG agar contain selective and differential ingredients (oxgall and brilliant green in EE broth, and bile salts #3 and crystal violet in VRBG agar) that may prevent resuscitation of injured *E. sakazakii*, precluding its detection in powdered infant formula and other foods. Several media have been recently developed for detecting *E. sakazakii* in powdered infant formula. As promising as these media are for recovering *E. sakazakii* from powdered infant formula and other foods, their relative suitability for supporting resuscitation and colony development by cells after exposure to various stress environments has not been evaluated. We undertook a study with the objective to determine and compare the ability of eight agar media to resuscitate and support colony development by healthy and heat-, freeze-, acid-, alkaline-, and desiccation-stressed cells of *E. sakazakii*. Spiral plating and ecometric techniques were used to assess the performance of media.

Cells of four strains of *E. sakazakii* isolated from powder infant formula were exposed to five stress conditions: heat (55°C for 5 min), freezing (-20°C for 24 h. thawed, frozen again at -20°C for 2 h, thawed), acidic pH (3.54), alkaline pH (11.25),and desiccation in powdered infant formula ($a_w$ 0.25, 21°C for 31 days). Control and stressed cells were spiral plated on tryptic soy agar supplemented with 0.1% pyruvate (TSAP, a non-selective control medium), Leuschner, Baird, Donald, and Cox agar (LBDC, a differential non-selective medium), Oh and Kang agar (OK), fecal coliform agar (FCA), Druggan-Forsythe-Iversen medium (DFI), violet red bile glucose (VRBG) agar, and Enterobacteriaceae enrichment (EE) agar. With the exception of desiccation-stressed cells, suspensions of stressed cells were plated on these media and on R & F *Enterobacter sakazakii* chromogenic plating medium (RF) using the ecometric technique. The order of performance of media for recovering control and heat-, freeze-, acid-, and alkaline-stressed cells by spiral plating was TSAP > LBDC > FCA > OK, VRBG > DFI > EE; the general order for recovering desiccated cells was TSAP, LBDC, FCA, OK > DFI, VRBG, EE. Using the ecometric technique, the general order of growth indices of stressed cells was TSAP, LBDC > FCA > RF, VRBG, OK > DFI, EE. Results indicated that differential, selective media vary greatly in their ability to support resuscitation and colony formation by stressed cells of *E. sakazakii*. The order of performance of media for recovering stressed cells was similar using spiral plating and ecometric techniques but results from spiral plating should be considered more conclusive.

## *ENTEROBACTER SAKAZAKII* INFECTION IN NEONATAL MICE

(A. N. Richardson, S. Lambert, and M. A. Smith)

*Enterobacter sakazakii* has been associated with nosocomial infections in premature and very low birth weight human infants. The affected infants were exposed to *E. sakazakii* when fed with contaminated reconstituted powdered infant formula. In this study, experimental CD-1 suckling mice were challenged orally with reconstituted powdered infant formula inoculated with $10^9$ and $10^{11}$ CFU *E. sakazakii* strain MNW2 on postnatal day 3. Deaths occurring immediately or less than 15 hours post-treatment were suspected to result from gavaging technique and were not included in the analysis.

Twenty deaths occurred at least 15 hours post-treatment and were assumed to result from *E. sakazakii* infection. The remaining mice were euthanized and brains, ceca, and livers were excised and pooled in groups within each litter for culturing. *E. sakazakii* was isolated from brain, liver, and cecum tissues in animals treated with $10^{11}$ CFU as compared to brain and liver tissues in neonates administered $10^9$ CFU. *E. sakazakii* was not found to be present in control tissues. One out of three litters at $10^9$ CFU had neonatal deaths associated with *E. sakazakii* treatment whereas all litters (4/4) treated with $10^{11}$ CFU had at least three neonatal deaths. There was 17.8% lethality among pups administered $10^9$ CFU and 34.8% lethality among pups given $10^{11}$ CFU as compared to 0.0% lethality among control pups. *E. sakazakii* infection in neonatal mice may be similar to that in premature human neonates because of their underdeveloped CNS at full-term birth. Thus neonatal mice may potentially serve as a model for *E. sakazakii* infection in premature and very low birth weight human infants.

## SURVIVAL AND GROWTH OF *ENTEROBACTER SAKAZAKII* ON FRESH-CUT FRUITS AND VEGETABLES AND IN UNPASTEURIZED JUICE AS AFFECTED BY STORAGE TEMPERATURE

(H. Kim and L. R. Beuchat)

In recent years, the incidence of foodborne diseases associated with fresh produce has increased. During the decade preceding 1999, approximately 12% of foodborne illnesses in the U. S. have been linked to fresh fruits and vegetables. Bacteria belonging to the family Enterobacteriaceae have caused or been associated with outbreaks of foodborne illnesses implicating unpasteurized juice and fresh fruits and vegetables. Examples of these outbreaks include *Escherichia coli* O157:H7 infection linked to the consumption of lettuce and apple cider, salmonellosis linked to tomatoes and cantaloupe, and shigellosis linked to parsley. Outbreaks of *E. sakazakii* infections associated with fresh produce have not been documented. However, isolated *E. sakazakii* has been isolated from 8 out of 9 food factories and from 5 out of 16 households, and the organism has been isolated from lettuce and other vegetables. Because of its presence in the environment, there is a risk of contamination of fresh produce with *E. sakazakii*. Its ability to grow at temperatures as low as 5.5°C raises concern about survival and growth on fresh-cut produce and in unpasteurized juice at storage temperatures used at retail and in food service and home environments.

We did a study to determine the survival and growth characteristics of *E. sakazakii* on fresh-cut apple, cantaloupe, strawberry, watermelon, cabbage, carrot, cucumber, lettuce, and tomato and in juice prepared from these fruits and vegetables. Produce and juice were inoculated with *E. sakazakii* at populations of 2 - 3 $\log_{10}$ CFU/g and 1 - 2 $\log_{10}$ CFU/ml, respectively, and stored at 4, 12, or 25°C. Populations did not change or gradually decreased in fresh-cut produce and juice stored at 4°C but grew on fresh-cut apple, cantaloupe, watermelon, cucumber, and tomato and in all juices except apple, strawberry, cabbage, and tomato juice at 12°C. All fresh-cut fruits and vegetables except strawberry supported growth of *E. sakazakii* at 25°C. Growth occurred in all juices except apple, strawberry, and cabbage juice, followed by decreases in population to < 1 CFU/ml after 48 - 72 h, which coincided with decreases in pH and an increase in population of lactic acid bacteria. Increases in total counts occurred in all juices except strawberry juice stored at 25°C and apple and strawberry juice stored at 12°C. Total counts increased in cantaloupe, carrot, cucumber, and lettuce juice stored at 4°C. Populations of molds and yeasts increased in apple and tomato juice stored at 25°C but decreased to < 1 CFU/ml in cabbage, lettuce, and cucumber juice. Further characterization of the behavior of *E. sakazakii* on fresh produce and in unpasteurized juice as affected by commercial packaging and handling practices is warranted.

## SURVIVAL AND GROWTH OF *ENTEROBACTER SAKAZAKII* IN INFANT RICE CEREAL RECONSTITUTED WITH WATER, MILK, LIQUID INFANT FORMULA OR APPLE JUICE

(G. M. Richards, J. B. Gurtler, and L. R. Beuchat)

Documented cases of infection caused by *Enterobacter sakazakii* are rare, although they have been more frequent during the past two decades. The bacterium has been implicated most often in causing illness in preterm neonates, infants and children from 3 to 4 years of age, with at least 76 cases and 19 deaths of infants being reported. Cases of *E. sakazakii* infections in adults also have been reported. Conditions affecting survival and growth of *E. sakazakii* in reconstituted infant formulae have been described. Minimum growth temperatures were reported to be 5.5 - 8.0°C. Guidance and recommendations concerning control and elimination of *E. sakazakii* in powdered infant formulae and reconstituted formulae have been issued in a joint report by the Food and Agriculture Organization/World Health Organization. Reports that *E. sakazakii* has been isolated from rice and rice products and that infants and young children have been diagnosed with infection caused by the bacterium raises concern about its behavior in reconstituted infant cereals.

We undertook a study to determine the survival and growth characteristics of *E. sakazakii* in infant rice cereal reconstituted with various liquids. The influence of storage temperature on survival and growth was determined. A commercially manufactured dry infant rice cereal was reconstituted with water, apple juice, milk, or liquid infant formula, inoculated with a 10-strain mixture of *E. sakazakii* at populations at 0.27, 0.93, and 9.3 CFU/ml, and incubated at 4, 12, 21, or 30°C for up to 72 h. Growth did not occur in cereal reconstituted with apple juice, regardless of storage temperature, or in cereal reconstituted with water, milk, or formula and stored at 4°C. The lag time for growth in cereal reconstituted with water, milk, or formula decreased as the incubation temperature (12, 21 and 30°C) was increased. Upon reaching maximum populations of 7 - 8 $\log_{10}$ CFU/ml, in some instances populations decreased to nondetectable levels during subsequent storage which was concurrent with decreases in pH. *Enterobacter sakazakii,* initially at very low populations, can rapidly grow in infant rice cereal reconstituted with water, milk, or infant formula. Reconstituted infant rice cereal can support luxuriant growth of *E. sakazakii*. Reconstituted cereal that is not immediately consumed should be discarded or stored at a temperature at which *E. sakazakii* and other food-borne pathogens cannot grow.

## SURVIVAL AND GROWTH OF *ENTEROBACTER SAKAZAKII* ON FRESH PRODUCE AS AFFECTED BY TEMPERATURE AND EFFECTIVENESS OF SANITIZERS FOR ITS ELIMINATION

(H. Kim and L. R. Beuchat)

*Enterobacter sakazakii* is an emerging foodborne pathogen known to cause meningitis, sepsis, bacteremia, and necrotizing enterocolitis in preterm neonates and immunocompromised adults. This bacterium has been found in several types of foods, food processing plants, and the environment, although outbreaks of infection have been associated primarily with reconstituted, temperature-abused infant formula. While *E. sakazakii* has not been reported to cause illnesses linked to the consumption of fresh produce, it has been isolated from lettuce and other vegetables, thereby representing a potential risk to produce safety. We have observed that *E. sakazakii* can grow on several types of fresh-cut produce and in fruit and vegetable juices. 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. An objective of this study was to determine the survival and growth 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 onto the surface of apples, tomatoes, and lettuce.

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®) 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 $\log_{10}$ 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 $log_{10}$ CFU/apple. Reductions of ≥ 3.70 $log_{10}$ 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 $log_{10}$ 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 $log_{10}$ CFU/sample. Results provide insights to predicting survival characteristics of *E. sakazakii* on produce and the efficacy of sanitizers in killing the bacterium.