Research Note

Survival of Enterobacter sakazakii in a Dehydrated Powdered Infant Formula

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ABSTRACT

A quantity of dehydrated powdered infant formula was prepared to contain Enterobacter sakazakii strain 607 at approximately 10⁶ CFU/ml when rehydrated according to the manufacturer’s instructions. The survival of the microorganism in the dry formula was followed for 2 years, during which samples periodically were rehydrated and analyzed for viable E. sakazakii. During the initial 5 months of storage at room temperature, viable counts declined approximately 2.4 log cycles. During the subsequent 19 months, the concentration of viable E. sakazakii declined an additional 1.0 log cycle. These results indicate that a small percentage of E. sakazakii cells can survive for extended periods in dehydrated powdered infant formula.

During the past several years, Enterobacter sakazakii has received increased attention as a potential cause of meningitis, septicemia, or necrotizing colitis in infants, particularly neonates and infants with underlying chronic health conditions (1–4, 8, 9, 11–15, 17, 18). In several instances, the source of the microorganism has been powdered infant formula (3, 6, 9, 14, 15, 17, 18). Analysis of powdered infant formulas in the marketplace by various investigators has revealed E. sakazakii prevalence of 0 to 18%, depending in part on the size of the samples taken (10). The concentrations of the microorganism in the dehydrated formula have been almost always less than 1 CFU/100 g.

Dehydrated powdered infant formula is a microbiologically stable nonsterile product that is packaged in sealed containers. As long as the package is not opened, the product remains usable for up to 2 years or until the manufacturer’s expiration date. The detection of E. sakazakii in marketplace samples and the association between E. sakazakii infections and consumption of powdered infant formula suggest that this microorganism can survive for extended periods in this class of dehydrated products. However, no quantitative data on the survival characteristics of this microorganism in powdered infant formula have been reported. Because of the availability of a batch of infant formula that had been inoculated with E. sakazakii for a study of the thermal resistance of this microorganism (7), the current study was undertaken to provide data on the survival of the microorganism over the course of almost 2 years.

MATERIALS AND METHODS

Microorganism. E. sakazakii strain 607, a clinical isolate (obtained originally from F. Khambaty, U.S. Food and Drug Administration, Washington, D.C.), was maintained and cultured as described by Edelson-Mammel and Buchanan (7).

Preparation of inoculated powdered infant formula. A 1,350-g portion of a commercial powdered infant formula was purchased from a local retailer and inoculated with E. sakazakii 607 as described by Edelson-Mammel and Buchanan (7). The concentration of inoculant was such that when initially examined there was approximately 10⁶ CFU/ml when the formula was rehydrated according to the manufacturer’s instructions. After inoculation, the formula was transferred to a 1-liter screw-cap Nalgene bottle (Nalgene Nunc International, Rochester, N.Y.) and stored at room temperature (20 to 22°C).

Determination of survival. Periodically, the bottle was opened under a biological safety hood, and triplicate 4.25-g samples were transferred to individual sterile screw-cap tubes. Each 4.25-g sample was rehydrated by adding 30 ml of sterile room-temperature water, resulting in reconstituted formula with a powder-to-water ratio equivalent to that suggested by manufacturer.

All rehydrated samples and their appropriate dilutions were plated onto duplicate tryptic soy agar plates using a spiral platter (Spiral Biotech, Bethesda, Md.). The plates were incubated for 18 to 20 h at 36°C, and bacteria colonies were counted with the automatic plate counter (Spiral Biotech). D-values were calculated by linear regression using commercial spreadsheet software.

Surviving colonies were confirmed as E. sakazakii using the API 20E system (bioMérieux SA, Marcy l’Etoile, France). For every other sampling time, one of the distinctive yellow colonies was taken from one of the duplicate plates for two of the three sample replicates. The picked colony was placed in 5 ml of a sterile 0.5% NaCl solution. The solution was then used to inoculate an API strip. The strip was incubated, evaluated, and interpreted according to the manufacturer’s instructions. The resultant profile was then compared with those in a database supplied by the test manufacturer.

Determination of a_w. The water activity (a_w) of the powdered infant formula immediately upon opening and at the end of
The infant formula product in this experiment had a somewhat higher $a_w$ than did material assayed immediately after opening, i.e., 0.14 versus 0.27, respectively. This difference probably is due to a combination of the small volume of water used in inoculating the powdered infant formula and the water vapor acquired by the dehydrated formula during the short time the jar was open for sample removal. Whether this difference in $a_w$ would have any significant impact on the survival of *E. sakazakii* in powdered infant formula will require further research. However, the survival of bacteria in a dehydrated state is usually enhanced when the $a_w$ is very low. Powdered infant formula is packaged under an inert atmosphere to prevent nutrient oxidation, a condition that also can foster survival of dormant bacterial cells. These facts suggest that survival of *E. sakazakii* in hermetically sealed containers is likely.

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**REFERENCES**