

S7-1

Survival of *Enterobacter sakazakii* Attached to or in Biofilms on Stainless Steel Under Various Storage Temperatures or Relative Humidities

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Enterobacter sakazakii infections in neonates have been associated with consumption of reconstituted powdered infant formula (Van Acker *et al.*, 2001 CDC, 2002). Powdered infant formula has very low a_w which may inhibit bacterial growth, but *E. sakazakii* is tolerant of low a_w . In addition, *E. sakazakii* is able to form biofilms on surfaces, which enhances its resistance against environmental stresses.

Objectives

The survival of *E. sakazakii* attached to stainless steel coupons held at 4, 25, and 37°C and 43% relative humidity (RH) was studied. We also determined maturation curves of biofilm formed by *E. sakazakii* in M9 and infant formula broth at 25°C for 6 days and survival in biofilms on stainless steel at 25°C and 23, 43, 68, 85, and 100% RH.

Methods

E. sakazakii suspended in water or reconstituted infant formula was deposited on stainless steel coupons, dried, and stored at 4, 25, and 37°C with 43% RH for up to 60 days. For the determination of biofilm maturation curves, stainless steel which *E. sakazakii* attached to were immersed in M9 and reconstituted infant formula at 25°C for 6 days and the populations were measured. Biofilms of *E. sakazakii* formed on stainless steel immersed in M9 medium or reconstituted infant formula for 6 days at 25°C were subsequently held at 25°C under 23, 43, 68, 85, and 100% RH for up to 6 weeks.

Results

Initial populations of *E. sakazakii* attached to stainless steel at 4, 25, and 37°C significantly decreased within 10, 3, and 1 day(s), respectively, but the pathogen remained viable for up to 60 days (Fig. 1).

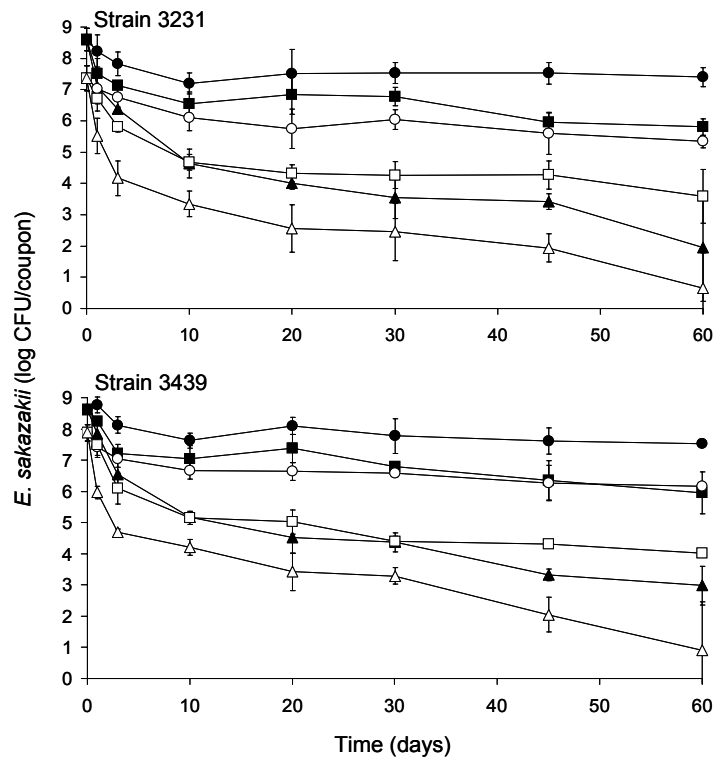


Fig. 1. Populations of *E. sakazakii* strains 3231 and 3439 suspended in sterile distilled water (open symbols) and reconstituted infant formula (closed symbols), inoculated and dried on the surface of stainless steel, and incubated at 4°C (circle), 25°C (square), and 37°C (triangle) for up to 60 days

Reductions were significantly greater when cells had been suspended in water rather than infant formula before drying.

This organism was observed not to form biofilms on stainless steel in lettuce juice or TSB at 12 or 25°C in our previous study (Kim *et al.*, 2006) but formed biofilms when immersed in M9 media and reconstituted infant formula (Fig. 2).

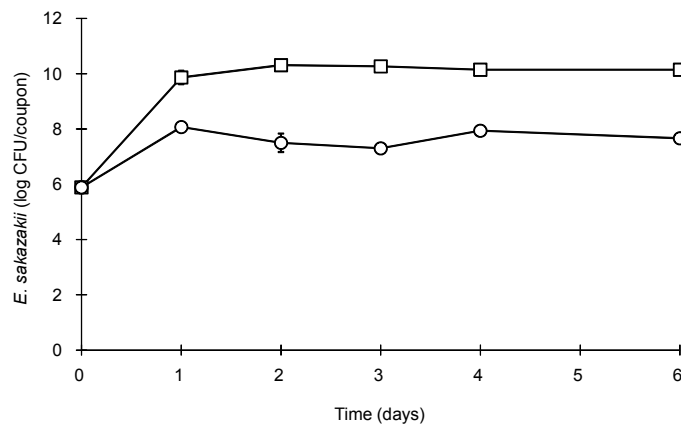


Fig. 2. Biofilm maturation curve of *E. sakazakii* strain 3231 when biofilms formed on stainless steel in M9 media (square) and infant formula broth (circle) at 25°C for 6 days.

Some cells in biofilms survived at 25°C under all test RHs for up to 6 weeks (Fig. 3). Populations of *E. sakazakii* in biofilm exposed to 85% RH decreased most rapidly. Reductions were significantly greater in biofilms that had formed in M9 medium compared to those formed in infant formula.

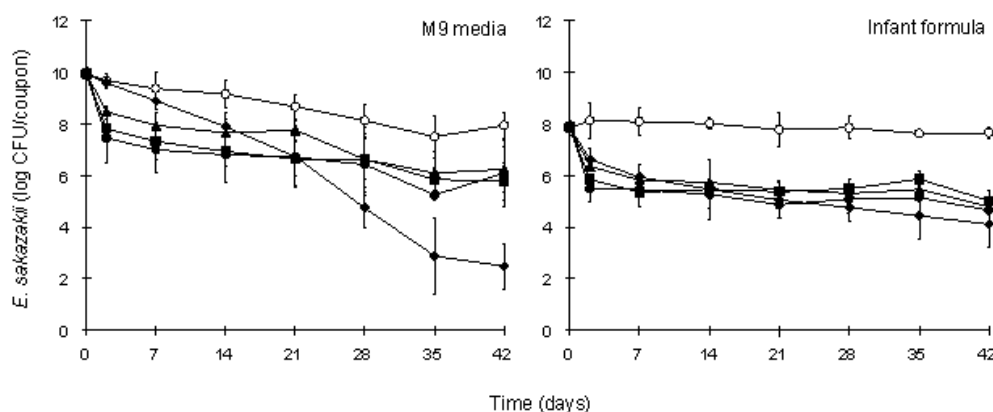


Fig. 3. Populations of *E. sakazakii* strain 3231 in biofilms formed on stainless steel in M9 media and infant formula for 6 days incubated at 25°C and 23 (●), 43 (■), 68 (▲), 85 (◆), and 100% (○) RH for up to 42 days.

Significances

Results indicate that infant formula provides protection for attached cells, as well as cells in biofilm, against desiccation stress. These results provide insights to predict survival of *E. sakazakii* on stainless steel, thereby providing insights to developing effective strategies and practices for elimination in processing and preparation kitchen environments.

References

- Centers for Disease Control and Prevention. 2002. *Enterobacter sakazakii* infections associated with the use of powdered infant formula-Tennessee, 2001. Morbid. Mortal. Weekly Rep. 51:297-300.
- Kim, H., J-H. Ryu, and L. R. Beuchat. 2006. Attachment and biofilm formation by *Enterobacter sakazakii* on stainless steel and enteral feeding tubes. Applied and Environmental Microbiology. 72:5846-5856.
- Van Acker, V., F. de Smet, G. Muyldermans, A. Bougatef, A. Naessens, and S. Lauwers. 2001. Outbreak of necrotizing enterocolitis associated with *Enterobacter sakazakii* in powdered milk formula. J. Clin. Microbiol. 39:293-297.