

ENTEROBACTER SAKAZAKII

ENTEROBACTER SAKAZAKII VIRULENCE BASED ON INFECTION IN NEONATAL MICE

(A.N. Richardson and M.A. Smith)

Enterobacter sakazakii (*E. sakazakii*) has been associated with outbreaks of infection in neonatal intensive care units (NICUs) among premature or very-low-birth-weight infants fed contaminated powdered infant formula. *E. sakazakii* infection can result in severe illnesses such as septicemia, meningitis, hydrocephalus, or even death. The objective of the research project is to develop an animal model to assess the ability of *E. sakazakii* to infect and cause disease in premature human infants. We used four mouse strains (CD-1, C57BL/6, BALB/C, and A/J) and exposed them to *E. sakazakii* strain MNW2 to determine which was the most susceptible to the infection. Timed-pregnant mice were obtained, acclimatized, and allowed to give birth naturally. At postnatal day (PND) 3 or 4, the pups were orally gavaged with a single dose of vehicle or 10^1 - 10^{11} CFU *E. sakazakii* per ml reconstituted powdered infant formula. Pups surviving to PND 10 or 11 were sacrificed and brains, livers, and ceca excised, pooled into groups within litters, and analyzed for the presence of *E. sakazakii*. The CD-1 mouse strain was the most susceptible showing the most infectivity and lowest infectious dose (10^2 CFU). We continued using CD-1 mice to observe differences in virulence among *E. sakazakii* strains MNW2, SK81, and 3290 and to compare the susceptibilities of male and female neonatal mice. On treatment day, the mouse pups were sexed and randomized so that each dam had a litter of ten (5 females and 5 males). At PND 10 or 11, surviving pups were sacrificed and individual tissues were excised and analyzed for *E. sakazakii* infection. Comparing the invasion of liver and brain tissues after treatment of pups with 10^5 CFU MNW2 and SK81 indicates that MNW2 is more invasive in both liver (43% versus 8%) and brain (71% versus 35%) than SK81. There appeared to be no significant difference in the susceptibilities of male and female CD-1 neonates administered *E. sakazakii* strain SK81, but the other strains have not been tested for sex differences. Testing of other strains of *E. sakazakii* is ongoing. Understanding and developing animal models for *E. sakazakii* infection will allow development of therapies to treat *E. sakazakii* infections. Comparisons of different strains of *E. sakazakii* will help determine which are more virulent and likely to cause morbidity and mortality in premature infants.

FATE OF ENTEROBACTER SAKAZAKII ATTACHED TO OR IN BIOFILMS ON STAINLESS STEEL UPON EXPOSURE TO VARIOUS TEMPERATURES OR RELATIVE HUMIDITIES

(H. Kim, J. Bang, L. R. Beuchat, and J.-H. Ryu)

Concerns about the occasional presence of *Enterobacter sakazakii* in powdered infant formula have surfaced as a result of reports of outbreaks of infections associated with consumption of reconstituted products. The bacterium may enter formulas via contaminated ingredients after spray drying of milk or soy components or by cross-contamination from the environment before packaging or during reconstitution in preparation areas. *E. sakazakii* has been observed to attach to or form biofilms on the surface of silicon, latex, polycarbonate, glass, polyvinyl chloride, and stainless steel. Cells that have attached to stainless steel and formed biofilms have enhanced resistance to disinfectants. *E. sakazakii* is reported to produce extracellular polysaccharides which may enhance the resistance of cells to environmental stresses such as in low a_w environments. Meager research attention has been given to characterizing the survival of cells of *E. sakazakii* attached to abiotic surfaces or in biofilm upon exposure to dry environments.

To develop effective strategies and practices for eliminating *E. sakazakii* in processing or preparation kitchen environments, factors affecting the survival of attached cells and cells in biofilm need to be better understood. We undertook a study to determine the survival characteristics of *E. sakazakii* cells suspended in water and reconstituted infant formula and dried on the surface of stainless steel as affected by subsequent incubation temperature at 43% relative humidity (RH) for up to 60 days. Maturation curves of biofilms formed in M9 medium and reconstituted infant formula, and survival of cells in biofilms formed in these media upon exposure to RH of 23 – 100% for up to 42 days were determined.

Initial populations of 7.4 - 8.6 log CFU/coupon decreased significantly ($p \leq 0.05$) at 4, 25, and 37°C within 10, 3, and 1 day(s), respectively, but the pathogen remained viable for up to 60 days. At a given storage temperature and time, reductions were significantly greater when cells had been suspended in water rather than infant formula before drying. Formation of biofilm by *E. sakazakii* on stainless steel immersed in M9 medium,

which contains minimal concentrations of nutrients, and infant formula at 25°C and subsequent survival of cells at 25°C as affected by exposure to 23, 43, 68, 85, and 100% RH were investigated. Some of the cells in these biofilms survived under all test RHs for up to 42 days. The overall order of survival as affected by RH was 100 > 23 = 43 = 68 > 85% RH, regardless of the medium in which the biofilm was formed. Reduction in viability of cells was significantly greater in biofilm that had formed in M9 medium than in biofilm formed in infant formula. Results indicate that infant formula provides protection for attached cells, as well as cells in biofilm, against lethality upon exposure to desiccation. These results are useful when predicting the survival characteristics of *E. sakazakii* on stainless steel, thereby providing insights to developing and applying effective strategies and practices for elimination of the pathogen in processing and preparation kitchen environments.

