

BIOFILMS

CULTURE AND DETECTION OF *CAMPYLOBACTER JEJUNI* WITHIN MIXED MICROBIAL POPULATIONS OF BIOFILMS ON STAINLESS STEEL (S.Q. Sanders, D.H. Booth, J.F. Frank, and J.W. Arnold)

The objective of this research was to observe the formation and composition of biofilms that contain *Campylobacter jejuni*. Biofilms containing natural populations of bacteria from the poultry processing environment and the target pathogen, *C. jejuni*1221gfp, were produced. Growth kinetics were observed at four cell densities to determine temporal compatibility with biofilm mixtures. Thus, a protocol was developed for growing *C. jejuni* within biofilms on stainless steel coupons. Analysis of the biofilms by confocal laser scanning microscopy showed that *C. jejuni*1221gfp formed a biofilm on stainless steel when incubated without other bacteria. The average surface area of steel covered by *C. jejuni*1221gfp increased from 24 h to approximately equivalent levels at 48 and 96 h. *C. jejuni*1221gfp and natural bacterial populations formed biofilms on stainless steel. This mixture was characterized by an initial increase and subsequent decrease of the surface area coverage of stainless steel by *C. jejuni*1221gfp for each time period. Data on the surface area of stainless steel associated with *C. jejuni*1221gfp when incubated with either of two different initial inoculum densities of other bacteria suggested that the presence of natural populations of bacteria enhanced the numbers of *C. jejuni* on stainless steel. This work provides the basis to study interactions of *C. jejuni* with other bacteria.

PROTEOMIC ANALYSIS OF A HYPOCHLOROUS ACID TOLERANT *LISTERIA MONOCYTOGENES* CULTURAL VARIANT EXHIBITING ENHANCED BIOFILM PRODUCTION (J.P. Folsom and J.F. Frank)

Following exposure of *Listeria monocytogenes* ScottA (SA) to hypochlorous acid, rough colony variants were identified that were tolerant of hypochlorous acid and produced increased amounts of biofilm. A derivative of one of these variants was smooth, produced even more biofilm and exhibited greater biofilm chlorine resistance. The objective of this research was to compare the protein expression of a cultural variant to SA, and identify proteins that may be associated with biofilm production and chlorine tolerance. Suspension chlorine tolerance for several cultural variants (SAR, SAR5, and SBS) was determined by exposure to 60-120 ppm hypochlorous acid for five minutes. Hypochlorous acid tolerance of biofilms was determined after growing biofilms on stainless steel followed by exposure to 200 ppm hypochlorous acid for 5 min. All cultural variants were able to survive 120 ppm of hypochlorous acid in suspension. There was little difference in the hypochlorous acid tolerance of the cultural variant planktonic cells. The cultural variants produced greater amounts of biofilm than the SA form, and were more hypochlorous acid tolerant. The SBS variant was selected for proteomic comparison because it was the variant that produced the most biofilm and was most tolerant of hypochlorous acid when grown as a biofilm. Protein expression of planktonic and biofilm cells of SBS was compared to SA by using two dimensional difference gel electrophoresis. The 50s ribosomal protein, L10 was down regulated in biofilm SBS. Other proteins down regulated in planktonic SBS were the peroxide resistance protein (Dpr) and a sugar binding protein (LMO0181). This sugar binding protein was also up regulated in biofilm SBS. One protein spot down regulated in planktonic SBS contained both 50s ribosomal protein L7/L12 and an unknown protein (LMO1888).

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.

