

BIOFILMS

CHLORINE RESISTANCE OF *LISTERIA MONOCYTOGENES* BIOFILMS AND RELATIONSHIP TO SUBTYPE, CELL DENSITY AND PLANKTONIC CELL CHLORINE RESISTANCE

(J.P. Folsom and J.F. Frank)

Strains of *Listeria monocytogenes* vary in their ability to produce biofilms. This research determined if cell density, planktonic chlorine resistance or subtype are associated with the resistance of *L. monocytogenes* biofilms to chlorine. Thirteen strains of *L. monocytogenes* were selected for this research based on biofilm accumulation on stainless steel and rep-PCR subtyping. These strains were challenged with chlorine to determine the resistance of individual strains of *L. monocytogenes*. Planktonic cells were exposed to 20 through 80 ppm sodium hypochlorite in 20 ppm increments for five minutes in triplicate per replication, and the experiment was replicated three times. The number of tubes with surviving *L. monocytogenes* was recorded for each isolate at each level of chlorine. Biofilms of each strain were grown on stainless steel coupons. The biofilms were exposed to 60 ppm of sodium hypochlorite. When in planktonic culture, four strains were able to survive exposure to 40 ppm of chlorine, while four strains were able to survive 80 ppm of chlorine in at least one of three tubes. The remaining five strains survived exposure to 60 ppm of chlorine. Biofilms of 11 strains survived exposure to 60 ppm of chlorine. No association of biofilm chlorine resistance and planktonic chlorine resistance was observed, however biofilm chlorine resistance was similar for strains of the same subtype. Biofilm cell density was not associated with chlorine resistance. In addition, biofilms that survived chlorine treatment exhibited different biofilm morphologies. This data suggests that chlorine resistance mechanisms of planktonic cells and biofilms differ, with planktonic chlorine resistance being more affected by inducible traits, and biofilm chlorine resistance being more affected by traits not determined in this study.

FORMATION OF BIOFILM AT DIFFERENT NUTRIENT LEVELS BY VARIOUS GENOTYPES OF *LISTERIA MONOCYTOGENES*

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Strains of *Listeria monocytogenes* exhibit a range of ability to form biofilms. The objectives of this study were to determine if genetically related strains exhibit similar biofilm-forming capacity, and the effect nutrient concentration has on the ability of different strains to produce biofilm. Biofilms of 30 strains of *L. monocytogenes*, obtained from a variety of sources, were grown on stainless steel in tryptic soy broth [TSB] or a 1:10 dilution of TSB [DTSB] for 24 hours at 32°C. The amount of biofilm formed was determined using image analysis after staining the cells with bisBenzimide H 33258 (Hoechst 33258). The strains were genetically subtyped by repetitive element sequence-based PCR (rep-PCR) using the primer sets rep-PRO_{D1} and rep-PRO_{G5}. Data were analyzed by using ANOVA and Duncan's multiple range test. Eleven strains produced the same amount of biofilm in the two media. Fourteen strains produced more biofilm in TSB than DTSB. Five strains produced more biofilm in DTSB than TSB. Serotype 4b strains produced more biofilm accumulation in TSB than serotype 1/2a strains, while serotype 1/2a strains produced more in DTSB than did serotype 4b strains. Growth in DTSB resulted in decreased biofilm accumulation for serotype 4b strains. There was no correlation between genetic subtype and the amount of biofilm accumulation. These results indicate that serotype 1/2a and serotype 4b strains differ in the regulation of their biofilm phenotype. The poor biofilm accumulation of serotype 4b isolates when grown in DTSB could be a factor in the predominance of serogroup 1/2 strains in food processing plants, where nutrients may be limited.

ATTACHMENT AND BIOFILM FORMATION BY *ENTEROBACTER SAKAZAKII* ON STAINLESS STEEL AND ENTERAL FEEDING TUBES

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Enterobacter sakazakii is a foodborne pathogen capable of causing meningitis, sepsis, bacteremia, and necrotizing enterocolitis in preterm neonates and immunocompromised adults. Powdered infant formula and milk powder have been implicated as vehicles in outbreaks of *E. sakazakii* infections. However, the pathogen also has been isolated from various clinical sources, food processing plants, the environment, lettuce, alfalfa sprouts, tomatoes, and other vegetables, cheese, minced beef, and sausage. Its presence in fresh produce raises the

possibility of this food group serving as a vehicle of the pathogen for infections in immunocompromised adults, particularly patients in hospitals and elderly adult assisted-care facilities. *E. sakazakii* has been reported to be able to attach to and form biofilms on silicon, latex, polycarbonate, stainless steel, glass, and polyvinyl chloride. Foods such as powdered infant formula and fresh produce represent potential vehicles of *E. sakazakii* infections in infants and immunocompromised adults, respectively. Contact of these and other foods containing the pathogen with abiotic or biotic surfaces could result in attachment and biofilm formation. Removal or inactivation of pathogens on inert surfaces in infant formula preparation areas and produce processing environments by washing with water or treating with disinfectants or sanitizers is not always achieved, possibly because cells are enmeshed in biofilms or otherwise protected against exposure to antimicrobials. Attachment and biofilm formation by *E. sakazakii* as affected by temperature and nutrient availability have been given only meager research attention.

We did a study to determine the effects of temperature and nutrient availability on attachment and biofilm formation by *E. sakazakii* on stainless steel and enteral feeding tubes. Five strains grown to stationary phase in tryptic soy broth (TSB), infant formula broth (IFB), and lettuce juice broth (LJB) at 12°C and 25°C were examined for the extent to which they attach to these materials. Higher populations attached at 25°C than at 12°C. Stainless steel coupons and enteral feeding tubes were immersed for 24 h at 4°C in phosphate-buffered saline suspensions ($7 \log \text{CFU/ml}$) to facilitate attachment of $5.33 - 5.51$ and $5.03 - 5.12 \log \text{CFU/cm}^2$, respectively, before immersing in TSB, IFB, or LJB and incubating at 12°C or 25°C for up to 10 days. Biofilms were not produced at 12°C. The number of cells of test strains increased by $1.42 - 1.67 \log \text{CFU/cm}^2$ and $1.16 - 1.31 \log \text{CFU/cm}^2$ in biofilms formed on stainless steel and feeding tubes, respectively, immersed in IFB at 25°C; biofilms were not formed on TSB and LJB at 25°C, indicating that nutrient availability plays a major role in processes leading to the accumulation of biometrics on the surfaces of these inert materials. These observations emphasize the importance of temperature control in reconstituted infant formula preparation and storage areas in preventing attachment and biofilm formation by *E. sakazakii*.

