Center for Food Safety, University of Georgia, Griffin, GA (http://www.griffin.uga.edu/cfs)
2004 Highlights

BIOFILMS

ATTACHMENT AND BIOFILM FORMATION BY *ESCHERICHIA COLI* O157:H7 ON STAINLESS STEEL AS INFLUENCED BY EXOPOLYSACCHARIDE PRODUCTION, NUTRIENT AVAILABILITY, AND TEMPERATURE

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Escherichia coli O157:H7 is known to produce extracellular polysaccharides (EPS). The presence of EPS surrounding cells provides a physical barrier to protect cells against environmental stresses. Exopolysaccharides are involved in various steps of biofilm formation, including development of a conditioning film, adhesion of cells, and the formation of microcolonies and a three-dimensional biofilm structure. The EPS matrix not only increases the resistance of cells in biofilms against environmental stresses but also facilitates availability of nutrients. Exopolysaccharide clearly acts as a conditioning film in the construction of the three dimensional structure of biofilm but there is some controversy concerning the role EPS plays in initial adhesion of bacteria to surfaces. There is evidence that EPS enhances as well as inhibits the initial attachment of microorganisms on inert surfaces. A reason for this discrepancy may be that EPS can act as an adhesive or antiadhesive, depending on the attachment surface and medium in which cells are suspended. The role EPS produced by *E. coli* O157:H7 plays in the initial attachment and biofilm formation by cells on stainless steel surfaces has not been described. The objectives of this study were to determine if EPS production by *E. coli* O157:H7 affects the initial attachment of cells on stainless steel surfaces and to determine the effects of nutrient availability and temperature in attachment and subsequent biofilm formation by wild-type and EPS-producing strains of *E. coli* O157:H7 on stainless steel coupons (SSC).

The influence of EPS production, nutrient availability, and temperature on attachment and biofilm formation by *E. coli* O157:H7 strains ATCC 43895 (wild type) and 43895-EPS (extensive EPS-producing mutant) was investigated. Cells grown on heated lettuce juice agar (HLJA) and modified tryptic soy agar (mTSA) were suspended in phosphate-buffered saline (PBS). SSC were immersed in the cell suspension (10^9 cfu/ml) at 4°C for 24 h. Biofilm formation by cells attached to SSC as affected by immersing in 10% tryptic soy broth (TSB), lettuce juice broth (LJB), and minimal salts broth (MSB) at 12 and 22°C was studied. A significantly lower number of strain 43895-EPS cells, compared to strain ATCC 43895 cells, attached to SSC during a 24-h incubation (4°C) period in PBS suspension. Neither strain formed biofilm on SSC subsequently immersed in 10% TSB or LJB but both strains formed biofilms in MSB. Populations of attached cells and planktonic cells of strain ATCC 43895 gradually decreased during incubation for 6 days in LJB at 22°C but populations of strain 43895-EPS remained constant for 6 days at 22°C, indicating that the EPS-producing mutant, compared to wild type strain, has higher tolerance to the low-nutrient environment presented by LJB. It is concluded that EPS production by *E. coli* O157:H7 inhibits attachment on SSC and reduced nutrient availability enhances biofilm formation. Biofilm formed under conditions favorable for EPS production may protect *E. coli* O157:H7 against sanitizers used to decontaminate lettuce and produce processing environments.

BIOFILM FORMATION BY *ESCHERICHIA COLI* O157:H7 ON STAINLESS STEEL AND ITS RESISTANCE TO CHLORINE AS AFFECTED BY EXOPOLYSACCHARIDE AND CURLI PRODUCTION

(J.-H. Ryu and L. R. Beuchat)

A biofilm can be defined as a sessile bacterial community of cells that live attached to each other and to surfaces. Attachment and biofilm formation by foodborne pathogens and spoilage microorganisms on food contact surfaces in processing plants are a public health and cross-contamination concern. Biofilms can also form on the surfaces of containers used for harvesting, transporting, and displaying foods at the

retail level and develop on food surfaces. *Escherichia coli* O157:H7 can form biofilm on stainless steel and sloughing of cells may result in cross-contamination of foods during processing. The resistance of bacterial cells embedded in biofilm against environmental stresses such as sanitizers routinely used in the food industry can be dramatically increased. *E. coli* O157:H7 has also been shown to produce curli, a thin, coiled fimbriae-like extracellular structure. Understanding the role of EPS and curli produced by *E. coli* O157:H7 on attachment, biofilm formation on foods and food contact surfaces, and protection of cells against sanitizers commonly used in processing plants and foodservice settings would provide fundamental information of practical significance when developing intervention strategies to eliminate or control the pathogen. The objectives of this study were to determine if the production of EPS and curli by *E. coli* O157:H7 affects attachment and biofilm formation on stainless steel, and to determine the influence of EPS and curli production on resistance of cells to chlorine.

The resistance of *E. coli* O157:H7 strains ATCC 43895-, 43895-EPS (an exopolysaccharide [EPS] overproducing mutant), and ATCC 43895+ (a curli-producing mutant), was studied. Planktonic cells of strains 43895-EPS and/or ATCC 43895+ grown under conditions supporting EPS and curli production, respectively, showed the highest resistance to chlorine, indicating that EPS and curli afford protection. Planktonic cells (ca. 9 $\log_{10}$ cfu/ml) of all strains, however, were killed within 10 min by treatment with 50 µg/ml chlorine. Significantly lower numbers of strain 43895-EPS, compared to strain ATCC 43895-, attached to stainless steel coupons but the growth rate of strain 43895-EPS on coupons was not significantly different than that of strain ATCC 43895-, indicating that EPS production did not affect cell growth during biofilm formation. Curli production did not affect the initial attachment of cells to coupons but did enhance biofilm production. The resistance of *E. coli* O157:H7 against chlorine increased significantly as cells formed biofilm on coupons; strain ATCC 43895+ was most resistant. Population size of strains ATCC 43895+ and ATCC 43895- in biofilm formed at 12ºC were not significantly different but cells of strain ATCC 43895+ showed significantly higher resistance compared to cells of strain ATCC 43895-. These observations support the hypothesis that the production of EPS and curli increase the resistance of *E. coli* O157:H7 to chlorine.