

Biofilm Formation of *Escherichia coli* O₁₁₁ on Plastic Surfaces

¹M.H. Movassagh Ghazani, ²J. Dolgharisharaf, ²M. Khajeh and ²K. Najafian

¹Department of Food Hygiene, Faculty of Veterinary Medicine, Islamic Azad University, Shabestar Branch, Iran

²Faculty of Veterinary Medicine, Islamic Azad University, Shabestar Branch, Iran

Abstract: 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 food-borne pathogens and spoilage microorganisms on food contact surfaces in processing plants are a public health and cross-contamination concern. Biofilms are found ubiquitously in virtually all natural, medical and industrial settings where bacteria exist. Biofilm formation by *Escherichia coli* O₁₁₁ on commonly used plastic surfaces was studied. For this study 12 plastic chips were used. *E.coli* strain was added to the beakers with TSB and the samples. *Escherichia coli* O₁₁₁ formed biofilm with a mean cell density of $7.69 \pm 0.19 \log \text{CFU/cm}^2$ on plastic surface. Based on the results, it can be concluded that *Escherichia coli* O₁₁₁ can survive on plastic surfaces. This is the first report, as far as we are aware, of biofilm formation by *Escherichia coli* O₁₁₁ on plastic surfaces. We were unable to find reports in our search of the literature.

Key words: Biofilm, *Escherichia coli*, plastic, surfaces

INTRODUCTION

Microbial biofilms are attracting attention of scientists in different areas such as the medical field, aquatic environment, food processing industries etc. Microbial biofilms may be detrimental and undesirable in food processing premises. Biofilms by pathogenic bacteria such as *Salmonella* (Dhir and Dodd, 1995; Humphery *et al.*, 1995; Jones and Bradshaw, 1996; Somers *et al.*, 1994), *Klebsiella* (Jones and Bradshaw, 1996; Morin *et al.*, 1996), *Pseudomonas* (Brown *et al.*, 1995), *Campylobacter* and enterohaemorrhagic *E. coli* O157:H7 (Somers *et al.*, 1994) and *Listeria* (Mafu *et al.*, 1990; Ren and Frank, 1993) have been reported. Such biofilms could be a continuous source of contamination to foods coming in contact with them when formed on contact surfaces.

This study was undertaken to understand the ability of *Escherichia coli* O₁₁₁ to form biofilms on plastic surfaces.

MATERIALS AND METHODS

Test organism: *Escherichia coli* O₁₁₁ strain PTCC 1270 (Iranian Research Organization for Science and Technology) was used.

Biofilm development: Plastic chips were used to develop the biofilm. Plastic chips (4 cm², commonly used in food processing equipment) were cleaned with detergent. For this study 12 plastic chips were used. Experiments were conducted wherein two samples of the plastic surface were placed in 1000 mL glass beakers and 200 mL of TSP (Scharlau, Spain) were added. *E.coli* strain was grown in TSB for 24 h at 37°C and 2 mL of this culture was added to the beakers with TSB and the samples. After incubation at 30°C for 48 h, the samples were aseptically removed, washed in sterile phosphate buffer saline (PBS, pH 7.4) to remove unattached cells and placed in beakers with TSB (Ren and Frank, 1993).

This procedure was repeated 5 times every alternate day to complete the biofilm formation.

Enumeration of biofilm cells: To enumerate biofilm cells after ten days of incubation, the samples were washed with sterile PBS to remove unattached cells and the biofilm cells were removed by swabbing with sterile cotton swabs. The swabs were transferred to 100 mL physiological saline (0.85% NaCl, w/v prepared in the laboratory) shaken vigorously and enumerated by standard spread plate technique. Tryptone soy agar (TSA, Scharlau, Spain) was used for enumeration and plates were incubated at 37°C for 48 h.

RESULTS AND DISCUSSION

Escherichia coli O₁₁₁ formed biofilm with a mean cell density of 7.69±0.19 log CFU cm⁻² on plastic.

Escherichia coli O₁₁₁ formed biofilms on plastic surfaces. The model system we studied indicates that the bacteria encountered in food processing environments can be very hardy and difficult to eliminate. Bacterial attachment and subsequent survival involve interactions between a bacterial cell, a surface and the surrounding microenvironment.

Scanning electron micrographs have also shown that food-borne pathogens and spoilage micro-organisms accumulate as biofilms on stainless steel, aluminum, glass, rubber and Teflon seals and nylon materials typically found in food-processing environments (Blackman and Frank, 1996; Czechowski and Banner, 1990; Herald and Zottola, 1988; Notermans *et al.*, 1991).

Helke (1993) showed that Milk and its components such as casein and b-lactoglobulin have also been found to inhibit the attachment of *Listeria monocytogenes* and *Salmonella typhimurium* (Helke *et al.*, 1993).

In the dairy industry, improperly cleaned and sanitized equipment (Czechowski and Banner, 1990; Koutzayiotis, 1992) and air-borne microflora (Schroder, 1984) are usually considered to be the major sources of contamination of milk and milk products. Cleaning-In-Place (CIP) procedures are usually employed in milk processing lines (Dunsmore, 1981; Dunsmore *et al.*, 1981). However, the limitation of CIP procedures is the accumulation of microorganisms on the equipment surfaces (Mattila *et al.*, 1990; Maxcy, 1964, 1969) resulting in biofilm formation.

CONCLUSION

Based on the results, it can be concluded that *Escherichia coli* O₁₁₁ can survive on plastic surfaces forming biofilm.

This is the first report, as far as we are aware, of biofilm formation by *Escherichia coli* O₁₁₁ on plastic surfaces. We were unable to find reports in our search of the literature.

ACKNOWLEDGMENT

The authors thank Dr. V. Deibel for her help in review of literature and Islamic Azad University, Shabestar Branch for partially funding this study.

REFERENCES

- Blackman, I.C. and J.F. Frank, 1996. Growth of *Listeria monocytogenes* as a biofilm on various food-processing surfaces. J. Food Prot., 59: 827-831.
- Brown, M.L., C.A. Henry and J.J. Gauthier, 1995. Relation between Glycocalyx and Povidone-Iodine resistance in *Pseudomonas aeruginosa* (ATCC 27853) biofilms. Applied Environ. Microbiol., 61 (1): 187-193.
- Czechowski, M.H. and M. Banner, 1990. Control of biofilms in breweries through cleaning and sanitizing. Tech. Q. Masters Brew. Assoc. Am., 29: 86-88.
- Dhir, V.K. and C.E.R. Dodd, 1995. Susceptibility of suspended and surface attached *Salmonella enteritidis* to biocides at elevated temperatures. Applied Environ. Microbiol., 61: 1731-1738.
- Dunsmore, D.G., 1981. Bacteriological control of food equipment surfaces by cleaning systems. I. Detergent effects. J. Food Prot., 44: 15-20.
- Dunsmore, D.G., A. Twomey, W.G. Whittlestone and H.W. Morgan, 1981. Design and performance of systems for cleaning product-contact surfaces of food equipment: A review. J. Food Prot., 44: 220-240.
- Helke, D.M., E.B. Somers and A.C.L. Wong, 1993. Attachment of *Listeria monocytogenes* and *Salmonella typhimurium* to stain less steel and Buna-N in the presence of milk and milk components. J. Food Prot., 56: 479-484.
- Herald, P.J. and E.A. Zottola, 1988. Scanning electron microscopic examination of *Yersinia enterocolitica* attached to stainless steel at elevated temperature and pH values. J. Food Prot., 51: 445-448.
- Herald, P.J. and E.A. Zottola, 1988. Attachment of *Listeria monocytogenes* to stainless steel surfaces at various temperatures and pH values. J. Food Prot., 53: 1549-1552, 1562.
- Humphery, T.J., E. Slater, K. McAlpine, R.J. Rowbury and R.J. Gilbert, 1995. *Salmonella enteritidis* Phage type 4 isolated more tolerant of heat, acid or hydrogen peroxide also survive longer on surfaces. Applied Environ. Microbiol., 61 (8): 3161-3164.
- Jones, K. and S.B. Bradshaw, 1996. Biofilm formation by the Enterobacteriaceae: A comparison between *Salmonella enteritidis*, *E. coli* and a Nitrogen fixing strain of *Klebsiella pneumoniae*. J. Applied Bacteriol., 80: 458-464.
- Koutzayiotis, C., 1992. Bacterial biofilms in milk pipelines. South African J. Dairy Sci., 24: 19-22.

- Mafu, A.A., D. Roy, J. Gonlet and P. Magny, 1990. Attachment of *Listeria monocytogenes* to stainless steel, glass, polypropylene, rubber surfaces after short contact times. *J. Food Prot.*, 53: 742-746.
- Mattila, T., A. Manninen and A.L. Kylasiurola, 1990. Effect of cleaning-in-place disinfectants on wild bacterial strains isolated from a milking line. *J. Dairy Sci.*, 57: 33-39.
- Maxcy, R.B., 1964. Potential microbial contaminants from dairy equipment with automated circulation cleaning. *J. Milk Food Technol.*, 27: 135-139.
- Maxcy, R.B., 1969. Residual microorganisms in cleaned-in-place systems for handling milk. *J. Milk Food Technol.*, 32: 140-143.
- Morin, P., A. Camper, W. Jones, D. Gatel and J. Goldman, 1996. Colonization and disinfection of biofilms hosting coliform colonized carbon fines. *Applied Environ. Microbiol.*, 62 (12): 4428- 4432.
- Notermans, S., J.A.M.A. Dormans and G.C. Mead, 1991. Contribution of surface attachment to the establishment of microorganisms in food processing plants: A review. *Biofouling*, 5: 1-16.
- Ren, T.J. and J.F. Frank, 1993. Susceptibility of starved planktonic and biofilm *Listeria monocytogenes* to quaternary ammonium sanitizer as determined by direct viable and agar plate counts. *J. Food. Prot.*, 56: 573-576.
- Schroder, M.J.A., 1984. Origins and levels of post pasteurization contamination of milk in the dairy and their effect on keeping quality. *J. Dairy Res.*, 51: 59-67.
- Somers, E.B., S.L. Schoeni and A.C.L. Wong, 1994. Effect of Trisodium phosphate on biofilm and planktonic cells of *Campylobacter jejuni*, *E. coli* O157:H7, *Listeria monocytogenes* and *Salmonella typhimurium*. *Int. J. Food Microbiol.*, 22: 269-276.