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Biofilm Formation of *Escherichia coli* O_{111} on Plastic Surfaces

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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* O_{111} 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* O_{111} formed biofilm with a mean cell density of 7.69±0.19 log CFU/cm^2 on plastic surface. Based on the results, it can be concluded that *Escherichia coli* O_{111} can survive on plastic surfaces. This is the first report, as far as we are aware, of biofilm formation by *Escherichia coli* O_{111} 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* O_{111} to form biofilms on plastic surfaces.

MATERIALS AND METHODS

Test organism: *Escherichia coli* O_{111} 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 cm^2, 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.

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RESULTS AND DISCUSSION

Escherichia coli O_{111} formed biofilm with a mean cell density of 7.69±0.19 log CFU cm^{-2} on plastic.

Escherichia coli O_{111} 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* O_{111} can survive on plastic surfaces forming biofilm.

This is the first report, as far as we are aware, of biofilm formation by *Escherichia coli* O_{111} on plastic surfaces. We were unable to find reports in our search of the literature.

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