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

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Abstract: Biofilms have been of considerable interest in the context of food hygiene. Of special significance is the ability of microorganisms to attach and grow on food and food-contact surfaces under favourable conditions. Biofilms are found ubiquitously in virtually all natural, medical and industrial settings where bacteria exist. Biofilm formation by *Escherichia coli* O_{111} on locally made cement surfaces was studied. For this study, 12 concrete chips were used. *E. coli* strain was added to the beakers with TSB and the samples. *E. coli* O_{111} formed biofilm with a mean cell density of 5.14±0.21 log CFU cm^2 on cement surface. Based on the results, it can be concluded that *Escherichia coli* O_{111} can survive on cement surfaces. This is the first report, as far as we are aware, of biofilm formation by *Escherichia coli* O_{111} on cement surfaces. We were unable to find reports in our search of the literature.

Key words: Biofilm, *Escherichia coli*, cement, microorganisms

INTRODUCTION

In nature and food systems, microorganisms get attracted to solid surfaces conditioned with nutrients, that are sufficient for their viability and growth. Microbial biofilms can form on virtually any surface that is in contact with water. 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 *E. coli* O_{111} to form biofilms on cement surfaces.

MATERIALS AND METHODS

Test organism: *Escherichia coli* O_{111} strain PTCC 1270 (Iranian research organization for science and technology) was used.

Biofilm development: The concrete chips were used to develop the biofilm. The concrete chips (4 cm^2, locally made) were scubbed with a brush and washed well with HPLC grade water. For this study, 12 concrete chips were used. Experiments were conducted wherein 2 samples of the concrete surfaces 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).

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 O_{111} formed biofilm with a mean cell density of 5.14±0.21 log CFU cm^2 on cement surfaces.

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Escherichia coli O_{111} formed biofilms on cement 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.

Ghazani *et al.* (2007) showed that *E. coli* O_{111} can survive on milk contact surfaces e.g., stainless steel and rubber surfaces forming biofilm. *E. coli* O_{111} formed biofilm with a mean cell density of 9.56±0.27, 9.10±0.15 log CFU cm^2 on milk contact stainless steel and rubber surfaces, respectively (Ghazani *et al*., 2007).

Ghazani *et al.* (2008) showed that *E. coli* O_{111} can survive on plastic surfaces forming biofilm. *E. coli* O_{111} with a mean cell density of 7.69±0.19 log CFU cm^2 on plastic surfaces.

Joseph *et al.* (2000) showed that *S. weltevreden* formed biofilm with a cell density of 6.19 log CFU cm^2 on cement surfaces.

Scanning electron micrographs have also shown that food-borne pathogens and spoilage microorganisms 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.

Based on the results, it can be concluded that *Escherichia coli* O_{111} can survive on cement surfaces forming biofilm.

Generally, an effective cleaning and sanitation programme, when included in the process from the very beginning, will inhibit both accumulation of particulates and bacterial cells on equipment surfaces and subsequent biofilm formation.

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

This study suggests that further research using sanitizers on cement surfaces and a physical method for the control of biofilms.

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