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# Biofilm Formation of *Escherichia coli* $O_{111}$ on Food Contact Stainless Steel and High Density Polyethylene Surfaces

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**Abstract:** Biofilm have been of considerable interest in the field of food hygiene. Microorganisms attach to surfaces and develop biofilms. Biofilm formation by *Escherichia coli* $O_{111}$ on commonly used surfaces viz stainless steel and high density polyethylene were studied. For this study, 12 stainless steel chips and 12 HDPE 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 $4.14 \pm 0.80$, $7.69 \pm 0.19$ log CFU/cm$^2$ on stainless steel and HDPE, respectively. There was significant difference ($p<0.05$) between bacterial counts of two type surfaces. Based on the results, it can be concluded that *E. coli* $O_{111}$ can survive on food contact surfaces e.g. stainless steel and HDPE surfaces forming biofilm.

**Key words:** Biofilm · Escherichia coli · Stainless steel · HDPE

## INTRODUCTION

A biofilm is an assemblage of surface-associated microbial cells that is enclosed in an extracellular polymeric substance matrix. Biofilms by pathogenic bacteria such as *Salmonella* [1-4], *Klebsiella* [3-5], *Pseudomonas* [6], *Campylobacter* and enterohaemorrhagic *E. coli O157:H7* [4] and *Listeria* [7,8] 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 potential food contact surfaces (Stainless steel and HDPE) and to compare both surfaces.

## MATERIALS AND METHODS

**Test Organism:** *E. coli* $O_{111}$ strain PTCC 1270(Iranian Research Organization for Science and Technology) was used.

**Biofilm Development:** Two types of material namely stainless steel and high density polyethylene (HDPE) were used to develop the biofilm. Stainless steel chips (4 cm$^2$, commonly used in food processing equipment, Iran Steel co, Iran ) were cleaned with acetone to remove grease and were etched by submerging in 5N HCl for 15 min, cleaned in detergent solution and finally rinsed in HPLC grade water. The HDPE chips (4 cm$^2$, commonly used in food processing equipment, Takplast, Iran) were cleaned with detergent. For this study 12 stainless steel chips and 12 HDPE chips were used. Experiments were conducted wherein two samples of the same type viz steel and HDPE were placed in 1000 ml glass beakers and 200 ml of Tryptic Soya Broth (Scharlau,Spain) were added. *E.coli* strain was grown in TSB for 24 hrs 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 hrs, the samples were aseptically removed, washed in sterile phosphate buffer saline (PBS, pH 7.4) to remove unattached cells and placed in beakers with fresh TSB[6].

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Table 1: Mean of *E. coli* $O_{111}$ biofilm population (log CFU/cm$^2$)

| Types of surface | Number of surfaces | Mean | Std Deviation | Std Error Mean |
|---|---|---|---|---|
| Stainless steel | 12 | 4.14 | 0.80 | 0.23 |
| HDPE | 12 | 7.69 | 0.19 | 0.05 |

Table 2: Result of t-test between bacterial counts of two type surfaces

| Types of surface | t | P value |
|---|---|---|
| Stainless steel and rubber | -14.831* | 0.002 |

*: Significant

This procedure was repeated five 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 hrs.

For the statistical analysis the SPSS computer program was used. The statistical significance between bacterial counts of two type surfaces was assessed by independent samples t test.

## RESULTS AND DISCUSSION

*E. coli* $O_{111}$ formed biofilm with a mean cell density $4.14 \pm 0.80$, $7.69 \pm 0.19$ log CFU/cm$^2$ on stainless steel and HDPE respectively. There was significant difference ($p<0.05$) between bacterial counts of two type surfaces (Tables 1 and 2). Biofilm variables are expressed log CFU per cm$^2$ of surface area.

*E. coli* $O_{111}$ formed biofilms on both stainless steel and HDPE surfaces. In fact the cell density/cm$^2$ was more on stainless steel surface compared to HDPE and there was significant difference ($p<0.05$) between bacterial counts of two type 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. Movassagh *et al.* [9,10] showed that *E. coli* $O_{111}$ formed biofilm with a mean cell density of $5.14 \pm 021$ and $5.03 \pm 014$ log CFU/cm$^2$ on cement and glass 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 [11-14]. Brading *et al.* [15] have emphasized the importance of physical forces in deatachment of biofilms.

Helke [16] 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*.

In the dairy industry, improperly cleaned and sanitized equipment [12,17] and air-borne microflora [18] 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 [19,20]. However, the limitation of CIP procedures is the accumulation of microorganisms on the equipment surfaces resulting in biofilm formation [21-23].

Based on the results, it can be concluded that *E. coli* $O_{111}$ can survive on food contact surfaces e.g. stainless steel and HDPE surfaces forming biofilm. Research on microbial biofilms is proceeding on many fronts, with particular emphasis on elucidation of genes specifically expressed by biofilm-associated organisms, evaluation of various control strategies for either preventing or remediating biofilm colonization of food contact surfaces.

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