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Biofilm Formation of *Escherichia coli* O_{111} on Food Contact Glass 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. Biofilm formation by *Escherichia coli* O_{111} on commonly used food contact glass surfaces were studied. For this study 12 glass chips(food grade) were used. *E. coli* strain was added to the beakers with Tryptic Soya Broth and the samples. *Escherichia coli* O_{111} formed biofilm with a mean cell density of 5.03 ± 0.14 log CFU/cm^2 on glass surfaces. Based on these results , it can be concluded that *Escherichia coli* O_{111} can survive on food contact glass surfaces forming biofilm . This is the first report ,as far as we are aware, of biofilm formation by *Escherichia coli* O_{111} on food contact glass surfaces. We were unable to find reports in our search of the literature.

Key words: Biofilm · *Escherichia coli* · Glass

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* [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 *Escherichia coli* O_{111} to form biofilms on potential food contact glass surfaces.

MATERIALS AND METHODS

Escherichia coli O_{111} strain PTCC 1270(Iranian Research Organization for Science and Technology) was used.

Glass was used to develop the biofilm. Glass chips (4 cm^2, commonly used for food packaging) 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. For this study 12 glass chips were used. Experiments were conducted wherein two samples of the same type viz glass 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 (Scharlau, Spain) 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 fresh TSB [8]. This procedure was repeated five times every alternate day to complete the biofilm formation.

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. For the statistical analysis the SPSS computer program was used.

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

Types of surface	Number of surfaces	Mean	Std Deviation	Std Error Mean
Glass	12	5.03	0.51	0.14

RESULTS

Escherichia coli O_{111} formed biofilm with a mean cell density of 5.03 ± 0.14 log CFU/cm^2 on glass (Table 1).

Biofilm variables are expressed log CFU per cm^2 of surface area.

DISCUSSION

Escherichia coli O_{111} formed biofilms on glass surfaces. The model system we studied indicates that the bacteria encountered in food processing environments can be very hardy and difficult o 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 *Escherichia coli* O_{111} formed biofilm with a mean cell density of 5.14 ± 0.21 and 7.69 ± 0.19 log CFU/cm^2 on cement and plastic surfaces, respectively. At this study the mean cell density of *Escherichia coli* O_{111} was lower than other type surfaces biofilms (cement and plastic).

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].

Helke [15] showed that Milk and its components such as casein and â-lactoglobulin have also been found to inhibit the attachment of *Listeria monocytogenes* and *Salmonella typhimurium*.

In the dairy industry, improperly cleaned and sanitized equipment [12,16] and air-borne microflora [17] 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 [18,19]. However, the limitation of CIP procedures is the accumulation of microorganisms on the equipment surfaces resulting in biofilm formation [20 -22].

Based on these results, it can be concluded that *Escherichia coli* O_{111} can survive on food contact surfaces e.g. glass surfaces forming biofilm. This is the first report, as far as we are aware, of biofilm formation by *Escherichia coli* O_{111} on food contact glass surfaces. We were unable to find reports in our search of the literature.

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