RESEARCH OPINIONS IN ANIMAL & VETERINARY SCIENCES

ISSN 2221-1896 (PRINT) www.roavs.com ISSN 2223-0343 (ONLINE)

Testing milk effect on formation of *Escherichia coli* O_{111} biofilm on stainless steel surface

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Abstract

In this study the effect of milk on biofilm formation by *Escherichia coli* O_{111} on commonly used stainless steel surface was studied. *Escherichia coli* O_{111} strain was added to the beakers with TSB and 12 stainless steel chips (treatment group) and ultra high temperature (UHT) milk. *Escherichia coli* O_{111} formed biofilms with a statistical mean cell density of 5.13±0.06 and 10.56±0.22 log CFU/cm^2 on stainless steel surfaces of control and treatment groups respectively. Based on the results, it can be concluded that the presence of milk enhanced the biofilm formation of *Escherichia coli* O_{111} on stainless steel surface.

Key words: Milk, Biofilm, *Escherichia coli*, Stainless Steel

Introduction

Milk is a highly perishable food which can be frequently in contact with stainless steel surfaces during its processing and storage (Barnes et al., 1999). Biofilms may comprise a single microbial species or multiple microbial species and can be formed on a range of biotic and abiotic surfaces (Armitage, 2005). The presence of microbial biofilms on the food contact surfaces is considered as a health hazard due to the fact that they may contain pathogenic microorganisms (Vlkova, 2008). According to Armitage (2005), biofilms grow in a three stage process. The initial stage includes the attachment of bacteria to the substratum. The bacterial growth and division then lead to the colonization of the surrounding area and the formation of the biofilm. Biofilms of pathogenic bacteria such as *Salmonella* spp (Dhir and Dodd, 1995; Humphery et al., 1995; Jones and Bradshaw, 1996; Somers et al., 1994), *Klebsiella* spp (Jones and Bradshaw, 1996; Morin et al., 1996), *Pseudomonas* spp (Brown et al., 1995), *Campylobacter* and enterohaemorrhagic *E. coli* O157:H7 (Somers et al., 1994) and *Listeria* spp (Mafu et al., 1990; Ren and Frank, 1993) have been reported. To control these problems, it has been recognized that a more understanding of the interaction between microorganisms and food-processing surfaces is required (Barnes et al., 1999).

This study was carried out to test the effect of milk on the adhesion of *Escherichia coli* O_{111} on stainless steel surfaces.

Materials and Methods

The used *Escherichia coli* O_{111} strain PTCC 1270 was used which was obtained from the Iranian Research Organization for Science and Technology. Twenty four stainless steel chips (4 cm square, 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. For control group, 12 samples of stainless steel chips were placed in 1000 ml glass beakers and 200 ml Tryptic Soya Broth (TSB) (Scharlau, Spain) were added. *E. coli* strain was grown in TSB for 24 hrs at 37ºC in a humidified incubator and 2 ml of this culture was added to the beakers with TSB and the samples.

For treatment group, 12 samples of stainless steel chips were placed in 1000 ml glass beakers, 200 ml of Tryptic Soya Broth (Scharlau, Spain) and 100 ml UHT milk (Mihan, Iran). After incubation at 30ºC in a humidified incubator for 48 hrs, the samples were washed in sterile phosphate buffer saline (PBS, pH 7.4) to remove unattached cells and placed again in beakers

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with fresh TSB and UHT milk. This procedure was repeated five times every alternate day to complete the biofilm formation (Brown et al., 1995).

To count 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 counted by standard spread plate technique. Tryptone soy agar (TSA, Scharlau, Spain) was used for counting and plates were incubated at 37 º C for 48 hrs (Joseph and Otta, 2001).

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

Results

Table 1 shows the density of *Escherichia coli* O_{111} cells formed on control and milk treated surfaces of stainless steel. There was significant difference ($P<0.01$) between the bacterial counts on the surfaces of the chips of the two group surfaces.

Table 1: *Escherichia coli* O_{111} cell count (log CFU/cm^2) of milk treated and control stainless steel

Group	Number of surfaces	Mean	SD	SE
Control	12	5.13^b	0.21	0.06
Experimental	12	10.56^a	0.76	0.22

Values bearing different superscripts in a column differ significantly ($P<0.05$) SD: Standard deviation, SE: Standard error

Discussion

The results indicated that milk has ability to enhance the biofilm formation of *Escherichia coli* O_{111} on stainless steel surface. This indicates that the bacteria encountered in food processing environments can be very hard and difficult to eliminate. Bacterial attachment and subsequent survival involve interactions between bacterial cell, surface and the surrounding microenvironment. Movassagh and Karami (2010) showed that *Escherichia coli* O_{111} formed biofilms of cell densities 5.14 ± 021 and 5.03 ± 014 log CFU/cm^2 on cement and glass surfaces respectively.

Food-borne pathogens and spoilage micro-organisms can 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). Brading et al. (1995) have emphasized the importance of physical forces in detachment of biofilms.

Helke et al. (1993) showed that milk and its components, such as casein and beta-lactoglobulin, have also been found to inhibit the attachment of *Listeria monocytogenes* and *Salmonella typhimurium* (Halke et al., 1993). According to the result of Barnes et al. (1999) skim milk was found to reduce adhesion of *E.coli* attached in very small numbers to stainless steel surfaces. This observation disagrees with our results of the current study in which whole milk was used for the biofilms formation and it could be the reason for this discrepancy.

Adhesion to the surface of milk treated stainless steel varied with the organism used (Barnes et al., 1999). They reported that the Gram positive *S. aureus* and *L. monocytogenes* cells and the Gram negative *S. marcescens* cells showed low density attachment (Barnes et al., 1999). They also noted that Gram negative *E. coli* and *P. fragi* cells adhered in small numbers to the clean stainless steel surface. It has previously been found that Gram negative organisms attached to stainless steel in larger numbers than gram positive organisms (Speers and Gilmour, 1985). Barnes et al. (1999) found that none of the milk proteins enhanced the attachment of *E. coli* to stainless steel surface. Austin and Bergeron (1995) observed that contact with milk solids is one of the reasons for low density attachment on the inside diameter of gaskets in milk processing equipments and this disagree with current results. Fletcher (1976) showed that serum albumin, gelatin, fibrinogen and pepsin inhibited bacterial attachment to petri dishes.

The present study suggests that the whole milk can augment attachment of *E.coli O111* to stainless steel surfaces.

Acknowledgment

The authors are grateful to Mr. J. Dolgharisharaf for assistance in conducting the laboratory work of this experiment.

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