Food Control 23 (2012) 389–396

Contents lists available at ScienceDirect

Food Control

journal homepage: www.elsevier.com/locate/foodcont

Prediction of *Escherichia coli* O157:H7 adhesion and potential to form biofilm under experimental conditions

Regina Célia Santos Mendonça*, Ana Márcia Faria Morelli [1], José Antonio Marques Pereira [1], Márcia Maria de Carvalho [1], Nádia Lopes de Souza [1]

Department of Food Technology, Viçosa Federal University, 36570-000 Viçosa, Minas Gerais, Brazil

ARTICLE INFO

Article history:
Received 8 March 2011
Received in revised form
26 July 2011
Accepted 2 August 2011

Keywords:
Predictive microbiology
E. coli O157:H7
Adhesion
Biofilm formation
Stainless steel
Poly(vinyl chloride) film

ABSTRACT

Escherichia coli O:157:H7 adhesion and potential to form biofilm on three different surfaces commonly used in the food industry was evaluated using probabilistic models; the surfaces tested were stainless steel 304 (SS304), poly(vinyl chloride) film covered with thick cloth (PVC1) and poly(vinyl chloride) film covered with thin cloth (PVC2). Using a Central Composite Rotational Design (CCRD), the effect of contact time (0 h, 7 h, 24 h, 41 h and 48 h) and temperature (12 °C, 17 °C, 28 °C, 39 °C and 44 °C) on the probability of achieving a particular adherent cell count (Log_{10} CFU cm^{-2}) was determined. By analyzing response surface plots and their corresponding contour plots and by determining quadratic equations for each surface, experimental values were shown to be significant in accordance with predicted values in all cases. The adjusted determination coefficient (R^2_{adj}) was 90.5%, 97.2% and 98.9% for SS304, PVC1 and PVC2, respectively, and the level of significance was $P \leq 0.001$. The bias factor (B_f) and accuracy factor (A_f) both approached 1.0 for the three surfaces evaluated. The model equations for predicting optimum response values were verified effectively by a validation data set for all surfaces evaluated. Therefore, an RSM provides a useful and accurate method for predicting *E. coli* O157:H7 adhesion and potential to form biofilm on SS304, PVC1 and PVC2 and could be considered to be a standard way to ensure food safety with respect to *E. coli* O157:H7 contamination through adhesion and biofilm formation.

1. Introduction

Escherichia coli O157:H7 (designated by its somatic, O, and flagellar, H, antigens) was first recognized as a human pathogen following two hemorrhagic colitis outbreaks in 1982. The first outbreak occurred in Oregon with 26 cases and 19 hospitalizations, and the second followed three months later in Michigan with 21 cases and 14 hospitalizations. Undercooked hamburgers from the same fast-food restaurant chain were identified as the vehicle, and *E. coli* O157:H7 was isolated from patients and a frozen ground beef patty. Outbreaks of *E. coli* O157:H7 infections have been primarily associated with eating undercooked ground beef, but a variety of other foods have also been implicated as vehicles (CDC, 2009). Cross-contamination of foods can occur in food-processing plants and during subsequent handling and preparation, resulting in a wide range of foods being implicated in outbreaks of *E. coli* O157:H7 infections. Recent studies have described an occurrence of *E. coli* O157:H7 infection due to milk consumption (Denny, Bhat, & Eckmann, 2008). *E. coli* serotype O157:H7 is a rare variety of *E. coli* but is a normal inhabitant of the intestines of all animals, including humans. The pathogen produces large quantities of one or more related potent toxins, called Shiga toxins, which cause severe damage to the lining of the intestine and to other target organs, such as the kidneys. The most severe outcome of Shiga toxin exposure among the general population is typically hemorrhagic colitis, a prominent symptom of which is bloody diarrhea. However, life-threatening complications sometimes occur. Some victims, particularly the very young, may develop hemolytic uremic syndrome (HUS). Overall, the Center for Disease Control and Prevention estimates that *E. coli* O157:H7 is responsible for approximately 73,500 infections, 2150 hospitalizations, and 61 deaths in the United States each year (CDC, 2009).

A biofilm can be defined as a sessile bacterial community of cells that live attached to each other and to surfaces, and its formation occurs on solid surfaces in contact with a liquid. Organic and inorganic material in the liquid can deposit as sediment on the solid surface. Subsequently, biologically active microorganisms attract to

* Corresponding author. Tel.: +55 31 3899 2293.
E-mail address: rmendonc@ufv.br (R. C. Santos Mendonça).
[1] Tel.: +55 31 3899 2230; fax: +55 31 3899 2208.

0956-7135
doi:10.1016/j.foodcont.2011.08.004

this conditioned surface and adhere to it. The microbial cells can then initiate growth, form an attachment matrix and develop into a complex community forming a microbial biofilm. The adhesion of bacterial cells in this manner to food-processing equipment can lead to a variety of problems, such as corrosion of metal surfaces and cross-contamination of processed foods; biofilms can also develop on food surfaces (Cooley, Miller, & Mandrell, 2003; Costerton, 1995; Gabis & Faust, 1988). While in a biofilm, the microbial community as a whole and specific individual cells exhibit increased resistance to sanitation measures and disinfectants, which makes it difficult to remove the biofilm or to inactivate particular microorganisms (Kumar & Anand, 1998). *E. coli* O157:H7 is known to produce exopolysaccharides (EPS), which can provide a physical barrier to protect cells against environmental stresses. EPS is also involved in cell adhesion and biofilm formation (Junkins & Doyle, 1992). EPS can serve as a conditioning film on inert surfaces, affect cell attachment by functioning as an adhesive or antiadhesive and influence the formation of three-dimensional biofilm structures (Ozer & Demirci, 2006; Ryu & Beuchat, 2005; Tamplin, Paoli, Marmer, & Phillips, 2005).

Predictive microbiology is a specific application of the field of mathematical modeling used to describe the behavior of pathogenic and spoilage microorganisms under a given set of environmental conditions. Growth-predictive models have been widely accepted as informative tools that can provide quick and cost-effective assessments of microbial growth for product development, risk assessment, and educational purposes. Although there are several classifications of predictive models, the classification proposed by Whiting and Buchanan (1993) (primary, secondary, and tertiary models) is currently the most commonly used.

In predictive microbiology, the development and application of secondary models for growth rate and lag time have been extensively reviewed (Devlieghere et al., 2000, 2001; McClure, Beaumont, Sutherland, & Roberts, 1997; Zurera-Cosano, Castillejo-Rodríguez, García-Gimeno, & Rincón-León, 2004). Square-root models describe the effect of suboptimal temperatures on growth rate. When this initial model is fitted to experimental growth rates, the data are square-transformed to stabilize their variance. This empirical relationship was transformed into a multiplicative model to consider the effects of additional environmental parameters such as CO_2, sodium lactate, or water activity (Dalgaard, Mejlholm, & Huss, 1997; Devlieghere et al., 2000, 2001). Some of the major advantages of these models are that they are simple, easy to interpret, and use few parameters. Furthermore, the biological significance of a microorganism's behavior can be obtained from the restricted parameters. Polynomial models were extensively used in the 1990s and are the most common secondary models. They do provide certain advantages; for example, it is easy to fit heterogeneous groups of experimental data using multiple linear regression techniques.

Response surface methodology (RSM), an empirical modeling technique used to estimate the relationship between a set of controllable experimental factors and observed results, is currently one of the most popular optimization techniques in the field of food science because of its comprehensive theory, reasonably high efficiency, and simplicity. The number of experimental points in the Central Composite Rotational Design (CCRD) is sufficient to test the statistical validity of the fitted model and the lack-of-fit of the model. Among secondary models, RSM has been commonly used in predictive microbiology to describe the effects of environmental dependences on the growth parameters of microorganisms. Similar to previous reports, RSM have been shown to successfully as a function of factors, such as pH, NaCl, temperature, and other preservatives (Buchanan & Phillips, 1993; McClure et al., 1997; Wijtzes, Rombouts, Kant-Muermans, Van't Riet, & Zwietering, 1993) for the growth of *Clostridium sporogenes* (Dong, Tu, Guo, Li, & Zhao, 2007) and *Leuconostoc mesenteroides* (Zurera-Cosano, García-Gimeno, Rodríguez-Pérez, & Hervás- Martínez, 2006), death of *Salmonella* Enteritidis (Koutsoumanis, Lambropoulou, & Nychas, 1999), growth rate and lag time of *Listeria monocytogenes* (Augustin & Carlier, 2000; Carrasco et al., 2006) and inactivation of *Listeria monocytogenes* (Gao, Ju, & Jiang, 2006) under different experimental conditions.

The objectives of this study were to describe *E. coli* O157:H7 adhesion and potential to form biofilm on stainless steel AISI 304, poly(vinyl chloride) film covered with thick cloth and poly(vinyl chloride) film covered with thin cloth at 12–44 °C for different contact times, and to develop and validate RSM using CCRD for mathematically predicting *E. coli* O157:H7 adhesion on these different surfaces commonly used by food industries.

2. Materials and methods

2.1. Bacterial strains and culture conditions

An aliquot of stock culture containing *E. coli* O157:H7 (strain ATCC 43895) kept at −25 °C in 15% (w/v) glycerol was inoculated in 10 mL of Nutrient broth (NB) and incubated for 48 h at 37 °C. Then, 10 mL of the culture was subcultured in 90 mL of NB, and the procedure was repeated for three consecutive days. A third subculture was grown for 18 h in 100 mL of NB until the early stationary phase was reached. All necessary dilutions were made in NB to obtain an inoculum size of 10^4 CFU mL^{-1}.

2.2. Surfaces used and chip preparation

Stainless steel AISI 304 #4 (SS304), poly(vinyl chloride) film covered with thick cloth (PVC1) and poly(vinyl chloride) film covered with thin cloth (PVC2) were selected as surfaces of study and used to prepare the chips. The chips were cut to dimensions of 10 × 10 × 1 mm.

All chips were cleaned with a neutral detergent solution for 20 min in an ultrasonic water bath (model 250D; VWR, West Chester, Pa.) and rinsed five times in 10 mL of sterile distilled water with agitation using a vortex. Cleaned chips were sterilized by UV exposure (100 mW cm^{-2}) for 60 min. In a subsequent step, the chips were degreased in ethylic alcohol for 1 h and rinsed twice with distilled water using a vortex. To finish, they were dried in a laminar air flow cabinet (Parizzi, Andrade, Silva, Soares, & Silva, 2004).

2.3. In vitro chip adherence assay

A cleaned and degreased chip was deposited in a glass flask containing the third subculture of *E. coli* O157:H7 adjusted to a concentration of 10^4 CFU mL^{-1} in NB, and then maintained at room temperature (25 °C). A chip was removed from the glass flask using sterile tongs and rinsed in 10 mL of 0.1% peptone water for 1 min to remove planktonic cells. Following this, the chip was transferred to a tube containing 5 mL of 0.1% peptone water and rinsed for 1 min with shaking using a vortex (model QL-901, Biomixer®, maxima potency: 2800 rpm) to remove attached cells (Parizzi et al., 2004). The cell adhesion to each surface was analyzed after 0 h, 2 h, 4 h, 6 h, 8 h and 10 h of contact.

The number of bacteria that adhered to each chip was evaluated using the plate count method. The number of CFU recovered from each chip was determined by plating appropriate serial dilutions in 0.1% peptone water on MacConkey agar plates and incubating them at 37 °C for 48 h. Each colony counted on a plate was equivalent to 2.5×10^2 CFU cm^{-2}, which was obtained by multiplying the diluent volume (5 mL) by the plated aliquot (1 mL) and dividing by the total surface area of the chip (2 cm^2). Time zero (0 h) corresponds to