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A predictive model for heat inactivation of *Listeria monocytogenes* biofilm on buna-N rubber

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Abstract

The purpose of this study was to develop a predictive model for the heat inactivation of *Listeria monocytogenes* in monoculture (strains Scott A and 3990) and with competing bacteria (*Pseudomonas* sp. and *Pantoea agglomerans*) formed on buna-N rubber with and without the presence of food-derived soil. Biofilms were produced on rubber disks in dilute Tryptic Soy broth (dTSB) with incubation for 48 h at 25 °C. Duplicate biofilm samples were heat treated for 1, 3, 5, and 15 min at 70, 72, 75, 77 and 80 °C and tested for survivors using enrichment media. The experiment was repeated six times. A predictive model was developed and plots were generated showing the percent probability of *L. monocytogenes* inactivation in biofilms after heat treatment. For example, to achieve a 95% probability level of complete inactivation required heat treatment of 76 °C for 6 min. The predicted model was validated using a five-strain cocktail of *L. monocytogenes*. The validated prediction model indicates that with proper maintenance of the time/temperature controls *L. monocytogenes* in biofilms on rubber surfaces will be inactivated. This model can be used as a tool in the selection of hot water sanitation processes for rubber surfaces.

Keywords: *Listeria monocytogenes*; Biofilm; Heat; Buna-N rubber

1. Introduction

Gaskets in food processing equipment are often made from buna-N rubber, also known as Nitrile butyl rubber (Storgards, Simola, Sjoberg, & Wirtanen, 1999a, b). Repeated cleaning and sanitation of buna-N rubber ages the surface leading to the development of cracks. These deteriorated surfaces are difficult to clean creating a favorable environment for food residue accumulation, and biofilm formation (Storgards et al., 1999a, b).

The attachment and biofilm formation of *Listeria* and other microorganisms are influenced by the physico-chemical properties of the surface such as surface charge, hydrophobicity, pH, temperature and nutrient composition of the preconditioning menstrum (McGuire & Krisdhasima, 1991; Helke, Somers, & Wong, 1993; Smoot & Pierson, 1998; Wong 1998). Surface materials used in food processing allow differing degrees of biofilm formation (Helke & Wong, 1994; Blackman & Frank, 1996; Storgards et al., 1999a, b), for example, *Listeria* and *Salmonella* were found to adhere more to hydrophobic surfaces than to hydrophilic surfaces although the attachment may be weak (Sinde & Carballo, 2000). The presence of food residue on buna-N rubber can affect microbial attachment and biofilm formation. Milk and milk components inhibited attachment of *L. monocytogenes* and *S. typhimurium* on buna-N rubber (Helke et al., 1993), while cream and fat increased biofilm formation of some *Bacillus* and *Pseudomonas* species (Storgards et al., 1999a, b). Buna-N rubber exhibited inhibitory effects on the growth and attachment of microorganisms including *L. monocytogenes* and *Pantoea agglomerans* (Ronner & Wong, 1993; Storgards et al., 1999a, b). Much information is available on the attachment, growth and biofilm formation

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of environmental and foodborne bacteria and the efficacy of chemical cleaning and sanitation used for their control. However, there are limited data available on the effect of hot water sanitation on the inactivation of bacterial biofilm in the food processing environment.

The objective of this research was to develop a predictive model for the heat inactivation of *L. monocytogenes* in monoculture and in biofilms with competing bacteria on rubber surfaces and in the presence of soil.

2. Materials and methods

2.1. Surface preparation

Buna-N rubber disks (1 cm^2) (McMaster-Carr, Atlanta, GA) were heated (100 °C) in a flask with 2 g/l alkali detergent (Micro™, International products corp., Burlington, NJ) then washed for ten cycles in an automatic dishwasher using alkali detergent (Jet Clean, Fisher Scientific, Pittsburgh, PA) to age the surface of the disks and to remove chemical residues. Disks were then sonicated (Aquasonic, model 550HT, VWR Scientific, Atlanta, GA) for 40 min in an alkali detergent (Micro™) at 50 °C rinsed three times in deionized water, and then autoclaved in type I quality water.

2.2. Culture source

Microorganisms used in this experiment are listed in Table 1. Strains of *L. monocytogenes* isolated from different sources were selected based on their heat resistance and the degree of the biofilm production determined in preliminary studies. *L. monocytogenes* Scott A and 3990 were selected for use in the predictive model because they were the most heat resistant and were high biofilm producers. *L. monocytogenes* strainsYM96 and 303 the moderate biofilm producers and *L. monocytogenes* 17 which produced minimal biofilm were used in the validation study along with the Scott A and 3990 strains.

2.3. Biofilm preparation

Cultures were activated from frozen beads (Microbank™, Prolab diagnostics, Austin, TX) in Tryptic Soy Broth (Difco brand, Becton Dickson, Sparks, MD) with incubation for 18 h at 32 °C. Biofilms were produced by immersing rubber surfaces into 1:10 diluted Tryptic Soy Broth (dTSB) inoculated with the 1 ml/l inoculum (10^6 cells/ml) appropriate cultures. The monoculture inoculum of *L. monocytogenes* consisted of strains Scott A and 3990, respectively. *Pseudomonas* sp. and *P. agglomerans* were also used to produce monoculture biofilms. Two multispecies culture inocula were used the first consisted of four parts *L. monocytogenes,* Scott A and one part *Pseudomonas* spp and, the next consisted of eight parts *L. monocytogenes*, 3990, one part *Pseudomonas* sp. and one part *P. agglomerans* (Table 1). These combinations were selected based on preliminary data indicating the ability of microorganisms to grow and maintain a population in a biofilm. Following the 4 h attachment of cells to the rubber surface at 25 °C, the surface was rinsed with phosphate buffer (0.01 mol/l, pH 7.0), transferred to fresh dTSB and incubated for 48 h at 25 °C. Biofilms from this protocol were considered as a 'low-soil' condition.

2.4. Preparation of biofilm with soil

Soil was formulated by emulsifying rendered chicken fat (138 mg/ml), sterile chicken serum (211–338 mg

Table 1
Microbial cultures used in biofilm formation on rubber surfaces

Name of microorganisms	Serovars	Origin/source[a]
Listeria monocytogenes Scott A	4b	Human clinical
Listeria monocytogenes 3990	4b	Vecherin Mont'd or cheese
Listeria monocytogenes YM 96	1/2a	Monkey environment
Listeria monocytogenes 303	1/2a	Monkey clinical
Listeria monocytogenes 17	4b	Food processing plant environment
Pseudomonas spp M21		Food processing plant environment
Pantoea agglomerans		Food processing plant environment
		Codes
L. monocytogenes, Scott A		Scott A
L. monocytogenes, 3990		LM 3990 or 3990
L. *monocytogenes* Scott A + *Pseudomonas* sp.		LMPs
L. monocytogenes, 3990 + *Pseudomonas* sp. + *P. agglomerans*		LMPsP

[a]All obtained from the Center for Food Safety, Griffin, GA.