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Effect of Sanitizers on *Listeria* Biofilm on Contact Surfaces

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Abstract

The effectiveness of sanitizers on biofilms of *Listeria monocytogenes* (NCTC 1994) developed on stainless steel and plastic (high density polyethylene, HDPE) surfaces was studied. There was more biofilm formation on the surface of stainless steel than on plastic with the latter being more resistant to the sanitizers than the former. Upon exposure to 100 ppm hypochlorite and 10 ppm iodophor for five minutes, there was a three to four log decrease in counts on the stainless steel surfaces, while on plastic surfaces, the reduction was one to two log cycles. Hypochlorite was more effective than iodophor in inactivating biofilm cells grown on both the surfaces. Two hundred ppm hypochlorite for five minutes. completely inactivated the adherent microcolony cells on stainless steel surfaces but failed to do so on plastic surfaces. Total inactivation of planktonic cells of *L. monocytogenes* was achieved using permitted concentrations of hypochlorite (10 ppm) and iodophor (1 ppm) for five minutes.

Introduction

Listeria monocytogenes has been recognized as an important foodborne pathogen ever since an outbreak of listeriosis in Canada was linked to the consumption of contaminated coleslaw (Schlech et al. 1983). Several outbreaks of listeriosis have been subsequently related to contaminated foods (Farber and Peterkin 1991). This has led to the imposition of a "zero tolerance" for *L. monocytogenes* in "ready to eat" foods by the United States Food and Drug Administation (USFDA) which has been a great challenge to the food processing industries since *L. monocytogenes* is an environmental organism and is present in a variety of

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raw foods including milk, meat, vegetables and seafoods (Farber and Peterkin 1991; Johnson et al. 1990; Fuchs and Reilly 1992, Ben Embarek 1994; Jeyasekaran et al. 1996).

Formation of microbial biofilms on food contact surfaces is a matter of great concern for the food processing industries. Attachment of a bacterium to a solid substrate is usually followed by a microcolony formation which may surround itself with extracellular polysaccharide, glycocalyx. In addition to being a physical barrier, the glycocalyx is strongly anionic and serves to protect the microcolony. It has been noted that free living planktonic cells are much more vulnerable to exogenous deleterious agents such as antibiotics, detergents or biocides than the same cells in a biofilm (Costerton et al. 1987). Archer (1990) suggested that biofilm formation by *L. monocytogenes* in the food processing environment could be a great challenge for the food processing industries. The objective of this investigation was to evaluate the ability of *L. monocytogenes* to form biofilms on various surfaces and to study the susceptibility of biofilms to various sanitizers.

Materials and Methods

L. monocytogenes (NCTC) 1994 was used in this study. Stainless steel and plastic (HDPE) coupons about 60 cm^2 were used. Stainless steel coupons were cleaned, using acetone to remove grease, etched by submerging in 5 N HCl for 15 min, cleaned in detergent solution, and finally rinsed in Type I reagent grade water (Ren and Frank 1993). The HDPE coupons were cleaned using detergent solution and rinsed in Type I reagent grade water (Krysinski et al. 1992). Biofilm cells were grown as described by Frank and Koffi (1990) using low nutrient medium (2 g tryptic soy broth and 8 g glucose in 1 liter distilled water). Prepared stainless steel and plastic coupons were placed individually in 250 ml beakers containing 100 ml medium and sterilized at 121°C for 15 min. The medium was inoculated with 2 ml of a 24 h culture of *L. monocytogenes* in tryptic soy broth containing 0.6% yeast extract (TSBYE). After two days at room temperature (28 ± 2°C), the coupons were aseptically removed and washed in sterile phosphate buffered saline (PBS) to remove unattached cells and placed in beakers of fresh sterile medium. The procedure was repeated for five cycles during 10 days of growth.

To enumerate biofilm cells, the coupons were washed in sterile PBS and the cells were removed by rubbing with sterile cotton swab. The swab was transferred to 100 ml PBS, shaken vigorously and serial tenfold dilutions were plated on tryptic soy agar containing 0.6% yeast extract (TSAYE). To test the sensitivity of biofilm cells to sanitizers, the coupons were rinsed in PBS and dipped in hypochlorite solution containing 100 or 200 ppm available chlorine and iodophor solution containing 10 or 20 ppm for five minutes. Coupons were transferred to a neutralizing solution (0.01 M sodium thiosulphate) for 30 sec. The treated coupons were rinsed with PBS and cells were enumerated after swabbing as described above. The control coupons

were dipped in sterile tap water, washed, and processed like treated coupons.

Sensitivity of planktonic cells to sanitizers was tested as follows: 24 h culture of *L. monocytogenes* in TSBYE was centrifuged and cell pellet was suspended in the same volume of sterile saline. To this suspension, hypochlorite or iodophor was added to give a final concentration of 10 ppm and 1 ppm available chlorine or iodine respectively. At 5, 10, 15, and 20 min., aliquots were taken, diluted, and plated as described above.

Results

The amount of biofilm cells of *L. monocytogenes* formed on stainless steel coupon was $10^6/cm^2$, while on the plastic coupon, it was $10^5/cm^2$ (Table 1). After treatment with 100 ppm chlorine, the listerial counts on stainless steel coupon was reduced to $10^2/cm^2$, whereas on the plastic coupon the reduction was minimal ($10^4/cm^2$). At 200 ppm chlorine, complete inactivation of biofilm cells on stainless steel coupon occurred, but on the plastic coupon, it could bring about only three log units reduction. However, iodophor exhibited a different inactivation pattern. At 10 ppm iodine, the listerial counts on stainless steel coupon was reduced to $10^3/cm^2$ and at 20 ppm level, it could reduce to only $10^2/cm^2$. But on the plastic coupon, the reduction in biofilm cells was less with $10^4/cm^2$ at 10 ppm iodine and $10^3/cm^2$ at 20 ppm. On the other hand, treatments with sanitizers hypocholorite and iodophor at 10 ppm and 1 ppm, respectively were completely effective in inactivating 10^7/ml planktonic cells of *L. monocytogenes* (Table 2).

Table 1. Effect of sanitizers on biofilms of *L. monocytogenes*.

Treatments	Types of surface (*Listeria monocytogenes*/cm^2)	
	Stainless steel	Plastic
Sodium hypochlorite (100 ppm)	2.80×10^2	2.60×10^4
Iodophor (10 ppm)	8.80×10^3	9.60×10^4
Sodium hypochlorite (200 ppm)	ND*	7.20×10^2
Iodophor (20 ppm)	3.27×10^2	2.44×10^3
Tap water	5.28×10^5	1.45×10^5
Control	1.54×10^6	7.60×10^5

Table 2. Effect of sanitizers on planktonic cells of *L. monocytogenes*.

Types of sanitizers	*L. monocytogenes* counts/ml after various contact times (min.)				
	0	5	10	15	20
Sodium hypochlorite (10 ppm)	7.20×10^7	ND*	ND	ND	ND
Iodophor (1 ppm)	3.35×10^7	ND	ND	ND	ND

* ND – Not detected

Discussion

L. monocytogenes formed biofilms on both stainless steel and plastic coupons (Table 1). In fact the cell density/cm^2 was more on the stainless steel coupon compared to HDPE. Washing in tap water brought about a marginal reduction in cell numbers. Exposure to 100 ppm chlorine brought about a four log reduction in cell numbers on the stainless steel coupon, but the reduction was only a little over a log unit in the case of plastic coupons. Two hundred ppm chlorine completely killed biofilm cells on stainless steel coupons, while on plastic coupons, 7.20 x 10^2 cells/ cm^2 were still present after exposure to such a high dose of Cl_2 for 10 min. Exposure to 10 ppm iodophor brought about a three log reduction in cells on stainless steel coupon and less than a log unit reduction in cells on plastic coupon. Upon exposure to 20 ppm iodophor, the reduction in cell numbers was four log units on stainless steel coupons and two log units on plastic coupons (Table 1).

Results in table 2 show that planktonic cells of *L. monocytogenes* were highly sensitive to exposure to hypochlorite and iodophor. Five minute exposure to 10 ppm Cl_2 and 1 ppm iodine completely destroyed planktonic cells at a density of about 10^7/ml. These results show that *L. monocytogenes* biofilm is highly resistant to sanitizers commonly used in the food processing industries. The resistance was dependent on the surface on which the cells adhered to. Biofilm on HDPE surface was more resistant than on stainless steel (Table 1). Even 200 ppm Cl_2 was ineffective in completely eliminating biofilm on plastic. Compared to hypochlorite, iodophor was much less effective in killing biofilm cells on both plastic and stainless steel surfaces. The results presented here confirm the observation of Frank and Koffi (1990) that surface adherent growth of *L. monocytogenes* is associated with increased resistance to sanitizers. The results have a bearing on food processing industries.

Conclusion

Based on the results, it can be concluded that *L. monocytogenes* can survive on food contact surfaces, e.g. plastic crates or stainless steel tables forming a biofilm and such adherent cells may not be removed during the washing and sanitizing processes unless special attention is paid to the removal of biofilms.

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