Original Article

Radiation sensitivity of listeria monocytogenes Planktonic and biofilm-associated cells

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Abstract

Bio films of Listeria monocytogenes were formed on sterile glass slides in a coincubation apparatus, using inoculated tryptic soy broth, incubated at 37°C for 48 h. The planktonic and biofilm cultures were subjected to incremental gamma radiation doses (0, 0.5, 1.0, 1.5, 2.0 kGy) from a Cobalt-60 source. The D10 values were calculated from the linear regression model for the logarithm of the surviving fraction and irradiation dose. The survivors and surviving fraction of planktonic and bio film associated cells decreased with increased irradiation doses. The D10 value of L. monocytogenes planktonic cells (0.476KGy) was higher than that of biofilm-associated cells (0.379KGy) indicating bio film cells were more sensitive to ionizing radiation than planktonic cells. The antimicrobial efficacy of ionizing radiation is therefore preserved or enhanced in the treatment of biofilm-associated bacteria.

Keywords: Listeria monocytogenes, biofilms, planktonic radiation, sensitivity

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INTRODUCTION

Listeria monocytogenes is an important food borne pathogen[1] partly because of its high mortality rate of 20-40% in humans far exceeding other food borne infections[2] and also its association with a wide range of foods such as salads, sea foods, meat, and dairy products[3]. The risk of listeriosis associated with fruits and vegetables has been revealed to be high[4,5] especially when they are cut, sliced or shredded. Cut fruits and vegetables provide an increased surface area for contamination, growth and microbial attachment to an extent that they present a six to seven-fold increase in microbial numbers as compared to whole ones[6]. Conventional chemical sanitizers are commonly used to sanitize produce surfaces to reduce microbial contamination before consumption. However, the efficacy of conventional sanitizers to reduce contamination of attached microbes (biofilms) on fruits and vegetables has come under question. A biofilm is a community of cells irreversibly attached to either a biotic or abiotic surface enclosed in a complex exopolymeric substance (EPS)[7,8]. Biofilm formation is a survival strategy microbes adopt to enable them survive unpredictable environmental stressors such as temperature changes, desiccation, ultraviolet radiation, cleansing agents such as biocides and disinfectant pressure as well as host immune systems. Increased resistance (600-fold) of biofilm associated bacteria to biocides over that required to kill planktonic cells has been documented[9]. Listeria monocytogenes has been observed to be more resistant to disinfectants than other food pathogens such as Salmonella, Escherichia coli O157:H7 and Shigella and also grows at refrigeration temperature due to strong biofilm formation[10,11]. The risk of listeriosis therefore increases when fruits and vegetables are stored for longer periods at even 20C before consumption. Radiation processing has been shown to eliminate surface-associated and internalized bacteria from vegetables[12,13]. The radiation D10 values (the dose of radiation required to reduce population by 1 log10, or 90%) of biofilm-associated organisms in relation to their planktonic counterparts particularly of Listeria monocytogenes on vegetables has however, not been extensively investigated. The objective of the study was to investigate the relative susceptibilities of planktonic versus biofilm cells of Listeria monocytogenes on glass to gamma radiation.

MATERIALS AND METHODS

Inoculum preparation

A fresh cow milk isolate was obtained from the culture collection of the Microbiology Department of the Animal esearch Institute (CSIR) in Accra, Ghana. . This was maintained on Tryptone Soya Agar (TSA, Oxoid) slants at refrigeration temperature and reactivated periodically whenever required for use. For use as inoculums, a 24h culture of the isolate was prepared on a TSA plate and incubated at 370C. A suspension was then prepared from the 24h culture in 9 ml Tryptone Soya Broth (TSB) and adjusted to turbidity equal to 0.5 McFarland standards (approximately10^7 cfu/ml).

Biofilm formation

On glass slides Pre-cleaned glass microscope slides, 7.62×2.54 cm were wrapped in foil and sterilized by autoclaving at 121°C for 15 min. The slides were aseptically placed into 50 ml tubes containing 25 ml of sterile TSB after sterilization to form a biofilm co-incubation apparatus described by[14]. This was then aseptically inoculated with 0.5ml of fresh inoculums in TSB. Tubes were held upright in a rack and incubated at 37°C for 48 h.

Radiation sensitivity D^{10} studies

Samples of L. monocytogenes (planktonic cells and biofilm) cells in tubes were subjected to gamma irradiation from a Cobalt-60 source of the Ghana Atomic Energy Commission at a dose rate of 2.3405 k Gy/hr. The sample tubes were placed in a polypropylene rack on ice to maintain a cool temperature within the irradiator. The following doses of gamma radiation were applied (0, 0.5, 1.0, 1.5, 2.0KGy). Each dose experiment was done in duplicates and duplicate plates were also used for each dilution.

Enumeration of Survivors

Tubes were opened and surviving planktonic cells were enumerated by withdrawing a 1-ml aliquot of the liquid culture, serial diluting with TSB peptone and water spread-plating on TSA. In withdrawing the sample of liquid culture containing planktonic cells, extreme care was taken to avoid contacting either the glass slide or the sides of the culture tube. Biofilm-associated cells were isolated and enumerated according to the method of[14]. The microscope slide was carefully removed using sterile forceps gripping the clean, dry upper portion of the slide, rinsed with 10 ml of sterile saline to remove unattached cells. The remaining biofilm cells on the slide were then scraped into 25 ml of TSB in a fresh tube using a sterile cotton wool swab after which the swab stick was broken into the tube containing the slide, closed and vortexed at high speed for 3 minutes. I ml of the culture was drawn aseptically, serially diluted in TSB and pour-plated on TSA. Plates were incubated at 370C and colonies were counted at 24h and 48h using a colony counter.

Statistical Analysis

Microbial counts (cfu/g) were transformed into logarithms and mean values of the data were subjected to regression analysis to determine D10 values using Microsoft Excel (Microsoft, USA) Tables 1 and 2. The D^{10} value was calculated from the linear regression model for the log of the surviving fraction and irradiation dose where the D10 is the negative inverse slope of the semi-log plot: $\log^{10} N /N^0 \_ - 1/D^{10} \times D$[15] where, No = initial viable count; N = viable count after irradiation with dose D.

RESULTS AND DISCUSSIONS

L. monocytogenes strain used for this experiment expressed significant ability to attach and grow on glass surface following incubation at 370C in TSB. Ionizing radiation effectively reduced the populations of both planktonic cells and biofilm cells. Mean cell counts of survivors of planktonic cells and biofilm cells as well as the surviving fraction decreased after subjection to incremental irradiation doses (Tables 1 and 2). Figure1 shows the radioresistance curves of the planktonic cells and biofilm cells with the corresponding regression equations. The D10 of planktonic cells and biofilm cells were 0.476 and 0.379 kGy respectively. The linear correlation coefficients of the regression lines were all > 0.90 indicating a strong negative linear correlation.

Table 1: Survivors and surviving fractions of planktonic cells of Listeria monocytogenes irradiated with incremental doses.

Dose (KGy)	Surviving population (log10 cfu/ml)	Surviving Fraction ($\log_{10}N/N_0$)
0	9.71	0
0.5	8.40	-1.31
1.0	7.60	-2.11
1. 5.	6.32	2.39
2.0	5.00	4.71

Values are means of four replicates.

Table 2: Survivors and surviving fractions of bioflim cells of Listeria monocytogenes irradiated with incremental doses

Dose (KGy)	Surviving population (log10 cfu/ml)	Surviving Fraction ($\log_{10}N/N_0$)
0	6.52	0
0.5	4.85	-1.68
1.0	3.29	-2.23
1. 5.	2.10	-4.40
2.0	1.30	-5.22

Values are means of four replicates

In a comparison of the relative irradiation sensitivities of the two phenotypes, biofilm cells were more sensitive to irradiation than planktonic 0.476 KGy was needed in the case of planktonic cells. (Figure 1) The irradiation D^{10} values for Listeria planktonic cells (0.476KGy) and that for biofilm cells (0.379KGy) obtained in this study are comparable to D10 values for Listeria obtained in TSB for planktonic cells (0.374–0.926 kGy) and for biofilm-associated cells (0.380–0.682 kGy) by[12,4,16]. This study confirmed the biofilm

phenotype to be more sensitive to gamma radiation than the planktonic phenotype as was also reported by[14]. The implications of these results are in contrast with the relative efficacy of chemical sanitizers on planktonic versus biofilm-associated bacteria where the planktonic phenotype is more sensitive to sanitizers than biofilms. The primary mode of action of ionizing radiation is via oxygen and hydroxyl radicals[4]. In suspensions with a high antioxidant capacity, these radicals can be neutralized before doing damage to bacterial cell membranes, protein structures, and nucleic acid strands, thereby protecting the bacteria and reducing the efficacy of the process[16] The antimicrobial efficacy of the ionizing radiation against biofilm-associated pathogens observed in this study indicates that it is probable that neither the polysaccharide elements nor other associated elements such as DNA, proteins, etc., inhibited the generation of radical molecules throughout the biofilm, including within the resident bacteria. This study has demonstrated that ionizing radiation effectively reduces the populations of both planktonic and biofilm-associated L. monocytogenes. The study has further shown that, in contrast to chemical antimicrobial treatments, the antimicrobial efficacy of ionizing radiation is preserved or enhanced when treating biofilm-associated bacteria cells (Figure 1). Whereas 0.379 radiation was required to reduce the initial population of biofilm cells by 1 1-log cycle or 90%, 0. 476 KGy was needed in the case of planktonic cells. (Figure 1) The irradiation D10 values for Listeria planktonic cells (0.476KGy) and that for biofilm cells (0.379KGy) obtained in this study are comparable to D10 values for Listeria obtained in TSB for planktonic cells (0.374–0.926 kGy) and for biofilm-associated cells (0.380– 0.682 kGy) by[12 ,4,16]. This study confirmed the biofilm phenotype to be more sensitive to gamma radiation than the planktonic phenotype as was also reported by[14]. The implications of these results are in contrast with the relative efficacy of chemical sanitizers on planktonic versus biofilm-associated bacteria where the planktonic phenotype is more sensitive to sanitizers than biofilms. The primary mode of action of ionizing radiation is via oxygen and hydroxyl radicals[4]. In suspensions with a high antioxidant capacity, these radicals can be neutralized before doing damage to bacterial cell membranes, protein structures, and nucleic acid strands, thereby protecting the bacteria and reducing the efficacy of the process[16] The antimicrobial efficacy of the ionizing radiation against biofilm-associated pathogens observed in this study indicates that it is probable that neither the polysaccharide elements nor other associated elements such as DNA, proteins, etc., inhibited the generation of radical molecules throughout the biofilm, including within the resident bacteria. This study has demonstrated that ionizing radiation effectively reduces the populations of both planktonic and biofilm-associated L. monocytogenes. The study has further shown that, in contrast to chemical antimicrobial treatments, the antimicrobial efficacy of ionizing radiation is preserved or enhanced when treating biofilm-associated bacteria.

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