Hindawi
Journal of Food Quality
Volume 2018, Article ID 9141540, 6 pages
https://doi.org/10.1155/2018/9141540

Research Article

Effect of Temperature and Gamma Radiation on *Salmonella* Hadar Biofilm Production on Different Food Contact Surfaces

Najla Ben Miloud Yahia,[1] **Salma Kloula Ben Ghorbal**,[2] **Lobna Maalej**,[2] **Abdelwaheb Chatti**,[2] **Alya Elmay**,[3] **Nour-Eddine Chihib**,[4] **and Ahmed Landoulsi**[3]

[1]*Laboratoire de Biotechnologie et Techniques Nucléaires, Centre National des Sciences et Technologies Nucléaires, TechnoPole de Sidi Thabet, Sidi Thabet, Tunisia*
[2]*Laboratoire de Traitement et Valorisation des Rejets Hydriques, Centre des Recherches et des Technologies des Eaux (CERTE), Université de Carthage, Technopole Borj Cédria, BP 273, 8020 Soliman, Tunisia*
[3]*Laboratoire de Biochimie et de Biologie Moléculaire, Faculté des Sciences de Bizerte, Zarzouna, Tunisia*
[4]*CNRS, INRA, UMR 8207-UMET-PIHM, Université de Lille1, 369 rue Jules Guesde, CS 20039, 59651 Villeneuve d'Ascq, France*

Correspondence should be addressed to Najla Ben Miloud Yahia; najlabenmiloud@yahoo.fr

Received 11 December 2017; Accepted 20 February 2018; Published 28 March 2018

Academic Editor: Tomislava Vukušić

Salmonella is a pathogen transmitted by foods and it is one of the most important target bacteria in food irradiation studies. Few works were carried out on the effectiveness of gamma radiation against biofilms formed by this bacterium. *Salmonella* can form a biofilm on different material surfaces. The physicochemical properties of surfaces and environmental factors influence the adhesion of this pathogen. The present study investigated the effect of gamma radiation (1 and 2 kGy) and temperature (28°C and 37°C) on the development of *Salmonella* Hadar biofilm on polyvinyl chloride (PVC), glass, cellophane paper (CELLO), and polystyrene (POLY). The obtained results indicated that biofilm production is surface and temperature dependent. In addition, biofilm formation decreased significantly after gamma irradiation at either 1 or 2 kGy doses. However, the agfD and adrA genes expression did not demonstrate significant decrease. This work highlighted that gamma radiation treatment could reduce the biofilm formation of *Salmonella enterica* serovar Hadar on different food contact surfaces.

1. Introduction

Biofilm is known as sessile microbial communities attached to substances and interfaces and to each other [1]. Bacterial cells, in biofilm, are embedded into a matrix which forms a physicochemical barrier against different environmental conditions. The biofilm generation depends on substrate type (hydrophobicity), bacterial cells (flagellar formation and motility), and environmental conditions such as temperature that plays a crucial role in the phenotypic change from planktonic form to sessile one [2]. Hu et al. [3] indicated that Curli fibers (aggregative fimbriae) and agf genes (known as *csg*) are involved in biofilm formation. Also, bcsA, bcsB, bcsZ, and bcsC genes' coding for cellulose synthesis plays a crucial role in biofilm formation. Moreover, fimbriae production is coregulated by a LuxR-type regulator gene, agfD, acting indirectly on adrA gene for regulating cellulose production [4]. Several studies reported the ability of *Salmonella* spp. to develop biofilm on several materials (plastic, glass, stainless steel, and so on) and its resistance regarding antimicrobial agents under this physiological state [5, 6]. *Salmonella* spp. can produce biofilm on nutrient broth liquid-air interface [7, 8]. *Salmonella* spp. cultivated on Luria Bertani agar plates supplemented with Congo red express a phenotype known as "rdar" (red, dry, and rough) morphotype [7].

It was demonstrated that the agfD gene is necessary for the biofilm maturation and is responsible for all major matrix constituent's expression regulation [9]. Other studies indicated that this gene might be influenced by different stimuli [10].

Ionizing radiation (X-ray, gamma ray, and electron beam) is well-known as an effective method for destroying spoilage

and pathogenic microorganisms in foods [11]. The radiation of *D*10 values (dose of radiation necessary to reduce the population by 1 $\log_{10}$, or 90%) of various *Salmonella* strains was 0.65 kGy [12]. In addition, it was demonstrated that *D*10 value is depending on the isolates and substrate [13]. The first action of ionizing radiation is via oxygen and hydroxyl radicals, produced when the high-energy photons (X-ray and gamma) or electrons (electron beam) break water molecules [14]. These radicals harm cell membranes, protein structures, and nucleic acid strands. Irradiation also requires an orderly transfer of radiant energy into products and bacteria. We have previously described the effects of stress conditions (gamma radiation and static magnetic field) on *S*. Hadar. [13, 15]. In this work, we investigated the effect of ionizing radiation (gamma rays), temperature, materials, and the expression of agfD and adrA genes on *Salmonella* Hadar biofilm formation.

2. Material and Methods

2.1. Bacterial Strain and Growth Conditions. *Salmonella enterica* serovar Hadar was supplied by Pasteur Institute (Tunisia). It is a high antibiotic and radio resistant foodborne isolated from Turkish meat. *Salmonella* spp. were routinely cultivated on nutrient broth at 37°C for 24 hours.

2.2. Gamma Irradiation. Irradiation treatments were performed at the Tunisian semi-industrial Co_{60} gamma irradiation facility at a dose rate of 100 Gy/min. Doses were measured by the standard Fricke dosimeter. Irradiation was performed in 10 ml polyethylene tubes. Bacterial suspensions were irradiated at 1 and 2 kGy at room temperature (25°C). Control samples, nonirradiated, followed all the performed assays.

2.3. Biofilm Formation on Glass Tubes Assay. In order to investigate biofilm formation on glass tubes, pellicle formation was examined on an overnight culture incubated for 48 h at 28°C or 37°C, after gamma irradiation at 1 and 2 kGy doses according to the protocol proposed by Michal et al. [16].

2.4. Morphotypes on Congo Red Agar (CRA). The CRA method was done according to the protocol of Freeman et al. [17]. Media were prepared with Brain Heart Infusion (BHI) broth at 37 g/l, sucrose at 0.8 g/l, agar-agar at 10 g/l, and Congo red stain at 0.8 g/l. Plates of the medium were inoculated and incubated aerobically for 24 h at 37°C.

The biofilm positive strains produced black colored colonies and biofilm negative strains were pink colored [17].

2.5. The Microtiter Plate (MTP) Biofilm Assay. The MTP method was done according to the protocol of Møretrø et al. [18].

The biofilm production was carried out on different materials: polystyrene (POLY), polyvinyl chloride (PVC), glass (G), and cellophane paper (CELLO). For biofilm quantification, circular chips (1.3 cm diameter) of cellophane paper, polystyrene, and glass slides were used. These different materials were sterilized by autoclaving at 121°C for 15 min then placed into 96-well plates. Irradiated *Salmonella* (200 μl of bacterial suspension) was diluted 1 : 100 in TSB. Then 200 μl of this diluted suspension was transferred to each well. Plates were incubated for 24 h at 28°C and 37°C. After incubation, the broth was removed from each of the wells. Then, chips were transferred to a new 96-well microplate. Unfixed cells were removed by washing chips three times with phosphate buffer saline (PBS) and biofilm was dyed with crystal violet (1%). For colorimetric measurement, biofilm was resuspended in 200 μl of glacial acetic acid during 15 minutes and plates were read on ELISA plate reader multiscan (Microplaque reader 680, Biorad) at a wavelength of 595 nm. BHI medium was used as a negative control [19].

2.6. Quantification of Biofilm-Producing Genes in Salmonella. *Salmonella* spp. were inoculated into (BHI) and incubated at 28°C or 37°C for 48 hours. Following incubation, 1 ml of the bacterial suspension was transferred into microtubes and centrifuged at 10,000*g* for 10 minutes. The pellet was used for RNA experiments.

2.7. RNA Extraction and Quantitative Reverse Transcriptase PCR (qRTPCR). For total RNA extraction, Trireagent Total RNA Isolation System (Sigma) was used according to the manufacturer's protocol. RNA was suspended in 30 μl of sterile water after 15 minutes of incubation at 55°C. The final concentration measurement of RNA samples was performed using the NanoDrop ND1000 spectrophotometer (Thermo Scientific). RNA was extracted from both of irradiated and control samples. First strand cDNA synthesis was done using 1 μg total RNA in 25 μl reaction mixtures containing 10 mM deoxynucleoside triphosphate, 25 U of RNAsine, 5 μl of a 5x buffer, 50 U of AMV-RT (Promega), and 0.5 μg of random hexamers. The reaction mixture was incubated for 10 min at 25°C and then at 42°C for 30 minutes. Relative expression levels of *adr*A and agfD genes were fixed by RT-qPCR on cDNA achieved following the reverse transcription reaction. Amplification of both adrA and agfD genes was performed with primers as previously described by De Oliveira Débora et al. [20]. We used *recA* as reference gene for result normalization [21].

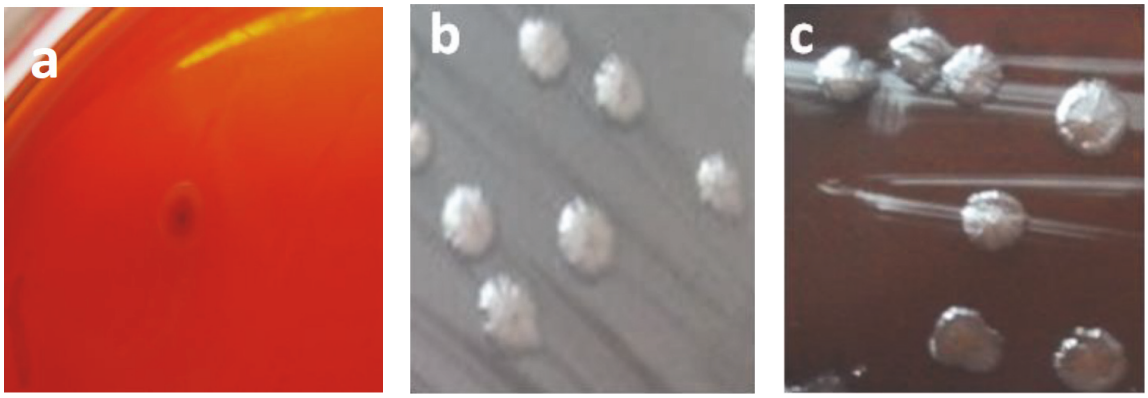
The qPCR amplifications were completed by mixing 10 ng cDNA with 12.5 μl mixture of SYBR green Supermix (Bio-Rad) and 300 nM primers.

The fluorescence signal was revealed by a Mini Opticon real-time PCR instrument (Bio-Rad). Data shown are the means of three replicates.

2.8. Statistical Analysis. Average values of triplicates were provided, and the deviation was less than 5% of each value. Significance was estimated at a level of $P < 0.05$ using Student's test.

3. Results

3.1. Salmonella's Colony Morphology and Biofilm Production. On CRA, untreated colonies appeared like black morphotype (Figure 1(a)). Concerning biofilm production assessed by microtiter plate (MTP), the results presented in Table 1 indicated that untreated *Salmonella* cultures preferentially adhere to PVC (O.D = 1.9) than to glass (O.D = 1.2). For



(a) (b) (c)

Figure 1: The morphotypes of *S.* Hadar before and after irradiation: (a) untreated black colonies; (b) bdar morphotype (1 kGy); and (c) rdar morphotype (2 kGy).

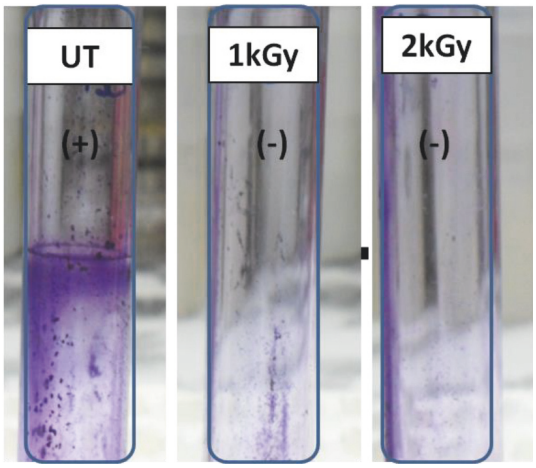


Figure 2: Biofilm production on glass tubes before (UT) and after gamma radiation at 1 kGy and 2 KGy doses. (−) indicates lack of pellicles and (+) indicates the presence of pellicles. UT: untreated bacteria.

the other materials (CELLO and POLY) adhesion is less important. Furthermore, for all the tested material biofilm production was enhanced at 28°C rather than at 37°C.

3.2. Effect of Gamma Radiation on Colony Morphology. For morphotype test, before treatment colonies were black (big biofilm producer) (Figure 1(a)). After gamma irradiation at 1 kGy, colonies appeared like bdar morphotype of blood agar (Figure 1(b)). However, at a dose of 2 kGy, the known morphotype, red, dry, and rough (rdar), was detected (Figure 1(c)), independently of the material pretested.

3.3. Effect of Gamma Radiation on Biofilm Formed Glass Surfaces. Biofilm production tested on the glass surface has demonstrated that, in the tube test, biofilm formation was considered as a ring of cells adhered to the glass wall at the air-liquid interface. Our results showed that untreated *S.* Hadar was able to produce biofilm on the air-liquid interface of glass tubes. Interestingly, we noted the absence of this ring after gamma irradiation (Figure 2). Moreover, biofilm formation using microplate wells with glass slides was significantly ($P < 0.05$) affected by gamma rays; the optical density reflecting biofilm biomass decreased from 1.5 (control) to 0.8 (1 kGy) and 0.1 (2 kGy) (Table 1). Therefore, the ability to form biofilm was solely dependent on the applied gamma irradiation dose.

Results for cellophane paper are very different and biofilm production was significantly ($P < 0.05$) reduced to 37% (from O.D = 0.9 to O.D = 0.5) at 1 kGy and up to 94% (from O.D = 0.9 to O.D = 0.07) at 2 kGy doses (Table 2). The biofilm formation of polystyrene material was inhibited by 95% at 2 kGy and at 28°C (Table 2).

3.4. Effects of Gamma Radiation on agfD and adrA Gene Expression. The agfD and adrA genes are widely dispersed in *Salmonella* genus and are always linked with the aptitude to produce biofilms [20]. Both of analyzed genes were detected in *Salmonella* Hadar. The expressions of biofilm encoding genes agfD and adrA were calculated with respect to recA as a reference gene. The expression of the genes agfD and adrA showed significant upregulation after gamma radiation (1 and 2 kGy). This increase seems to be independent of the temperature. However, a significant dose effect was observed at 37°C (Figure 3).

4. Discussion

Gamma irradiation is an established technology of well-documented safety and efficacy for inactivation of pathogenic microorganisms such as *Salmonella* [22]. However, studies into the effectiveness of irradiation on biofilm-associated cells are lacking.

Many bacteria form aggregates at the bottom of containers and attach to the container surfaces in liquid media. However, some bacteria such as *Salmonella*, *Escherichia coli*, *Pseudomonas fluorescent*, and *Vibrio cholera* produce rigid or fragile pellicle structures at air-liquid interfaces [23]. Biofilm production by colonization of the air interface can facilitate and contribute to gas exchange while enabling the acquisition

Table 1: *Salmonella* Hadar biofilm inhibition in presence of incremental gamma radiation doses.

Materials	Dose (gamma irradiation)	Morphotype on CRA	Test tube (glass)	$DO595_{(28^{\circ}C)}$	$DO595_{(37^{\circ}C)}$
PVC	Ut	Black	Nf	$1.9 \pm 0.00^{*}$	2.5 ± 0.08
	1 kGy	Bdar	Nf	$0.9 \pm 0.04^{*}$	1.3 ± 0.05
	2 kGy	Rdar	Nf	$0.08 \pm 0.00^{*}$	$0.1 \pm 0.01^{*}$
Glass	UT	Black	++	$1.2 \pm 0.02^{*}$	1.5 ± 0.06
	1 kGy	Bdar	+	$0.6 \pm 0.00^{*}$	$0.8 \pm 0.03^{*}$
	2 kGy	Rdar	+	$0.06 \pm 0.00^{*}$	$0.1 \pm 0.02^{*}$
Cello	UT	Black	Nf	$0.7 \pm 0.01^{*}$	$0.9 \pm 0.04^{*}$
	1 kGy	Bdar	Nf	$0.4 \pm 0.00^{*}$	$0.5 \pm 0.03^{*}$
	2 kGy	Rdar	Nf	$0.03 \pm 0.00^{*}$	$0.07 \pm 0.00^{*}$
Poly	UT	Black	Nf	$0.4 \pm 0.00^{*}$	0.6 ± 0.05
	1 kGy	Bdar	Nf	$0.2 \pm 0.02^{*}$	$0.2 \pm 0.04^{*}$
	2 kGy	Rdar	Nf	$0 \pm 0.00^{*}$	$0.04 \pm 0.00^{*}$

UT: untreated; Nf: not found; $^{*}P < 0.05$.

Table 2: Inhibition (%) of *Salmonella* Hadar biofilm formation, after gamma radiation exposure.

	PVC	Glass	CELLO	POLY
28°C				
1 kGy	54%	48%	37%	50%
2 kGy	96%	96%	95%	100%
37°C				
1 kGY	48%	50%	37%	57%
2 kGY	95%	94%	94%	93%

%: inhibition percentage.

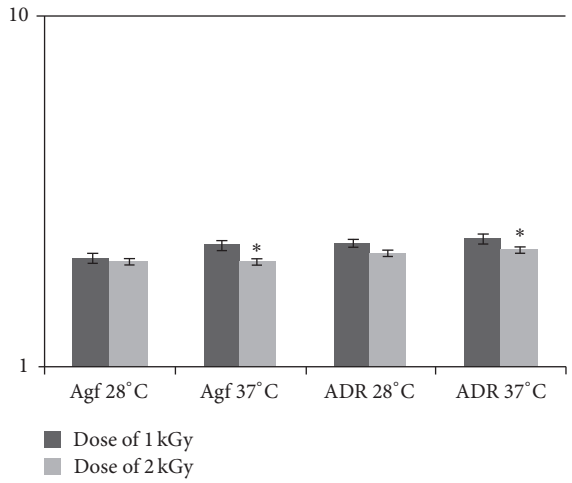


Figure 3: Effect of gamma radiation doses (1 and 2 kGy) on expression levels of agfD and adrA genes ($^{*}P < 0.05$).

of nutrients and water from the liquid phase [24]. The biofilms at air-liquid interfaces can cause severe problems in industrial water systems [25].

Slime production was evaluated by culture on CRA plates. Untreated colonies were black colored. Black pigmented colonies on Congo Red Agar indicate biofilm producer strains. The modification of agar ingredients can also improve phenotypic coloration [26]. Our results showed definite phenotypic changes following gamma radiation treatment at doses of 1 and 2 kGy. *S.* Hadar expressed bdar morphotype after 1 kGy treatment, then rdar morphotype was observed when treated by gamma radiation 2 kGy dose. Dubravka et al. [27] indicated that both rdar and bdar morphotypes are tolerant to disinfectants. Rdar morphology appears more tolerant to long-term *Salmonella* biofilm survival in a very dry environment, desiccation, and nutrient lack [28]. This result extends anterior results indicating that rdar morphotype indicates morphological adaptation to stress conditions and survival outside the host environment. The morphotype bdar has been suggested to be linked to *Salmonella* groups that do not need to survive for long periods in the host environment [29].

Biofilm production and adhesion on different surfaces were assessed and the same parameters described above were investigated such as (incubation temperature and gamma radiation doses). Bacterial adherence is influenced by the surface material, growth conditions, and the environmental factors including temperature [30]. These environmental factors play a vital role in the phenotypic change from planktonic cells to the sessile form [31].

Our results suggested that polystyrene is the material surface that presents the lower susceptibility to colonization. PVC avoided better the biofilm production at 37°C, followed by glass then cellophane. The high prevalence of biofilm production on PVC and glass at 37°C is agonizing because the permanency of *Salmonella* on these surfaces is in the starting of industrial processing and can be a valuable source of poultry contamination and a possible cause of foodborne diseases. Our results are in accordance with those reported by Hans et al. [4] who noticed that *Salmonella* adhered more easily to hydrophobic materials such as PVC than to stainless steel which is more hydrophilic. Mericarmen et al. [32] reported higher biofilm production by *Salmonella* on plastic than on stainless steel. Once a biofilm is formed, this could be a source of contamination for foods that is why protocols in food processing units should consider more *Salmonella*'s biofilm removal. Thus, all our findings highlight

the hypothesis that biofilm forming abilities could be reduced with temperature decrease and increasing gamma radiation doses. To our knowledge, such a linkage has not been reported previously for *Salmonella*. However, Alonso et al. [33] stated that gamma radiation was effective in reducing the populations of biofilm-associated cells of *Salmonella* enterica. Treatment with gamma irradiation at the end of the production chain can be a good solution for biofilm removal. No correlation was observed between agfD and adrA expression and bacterial biofilm production. However, it was found that in *Salmonella* CsgD (acting via agfD and adrA genes) altered cell physiology to enable the generation of Curli, a process not yet identified in *Salmonella* [34]. Zakikhany et al. [35] identified a CsgD independent cellulose pathway, adrA independent. Ben Abdallah et al. [36] showed that there is no correlation between sef and pef *Salmonella* genes involved in adhesion and invasion and biofilm formation. More considerable attention must be given to the choice of potential contact surfaces and cleaning procedures when considering the efficient removal of biofilms. Indeed, biofilm formation is strongly affected by different environmental signals via a complex regulatory network. Comprehensive overview must be given to the comprehension of this genetic network and the interactions between its various components (*CsgD, RpoS, Crl, OmpR, IHF, CpxR, mlrA, BarA/SirA, Csr, PhoPQ, RstA, Rcs*, metabolic process, and quorum sensing) [4].

In summary, this work showed that *Salmonella enterica* serovar Hadar could adhere and form the biofilm on industrial surfaces such as polystyrene, PVC, glass, and cellophane paper. This could be a factor to be considered for the higher spoilage or/and disease transmission. Our study revealed that the biofilm production is dependent on temperature and the abiotic surface used. Moreover, gamma rays could be considered as an effective mean for *Salmonella* biofilms removal. Finally, it was proposed that agfD and adrA genes were not actively involved in *Salmonella* biofilm production.

Conflicts of Interest

The authors have declared no conflicts of interest.

References

[1] N. Prasanna, R. Monica, D. Umer, U. K. Asad, Y. Aixin et al., "Biofilms in endodontics—current status and future directions," *International Journal of Molecular Sciences*, vol. 18, p. 1748, 2017.

[2] R. A. Rohit, A. a. Henrik, R. Olena, B. Nicolas, R. B. David et al., "A multivariate approach to correlate bacterial surface properties to biofilm formation by lipopolysaccharide mutants of *Pseudomonas aeruginosa*," *Colloids and Surfaces B: Biointerfaces*, vol. 127, pp. 182–191, 2015.

[3] L. Hu, C. J. Grim, A. A. Franco et al., "Analysis of the cellulose synthase operon genes, bcsA, bcsB, and bcsC in Cronobacter species: Prevalence among species and their roles in biofilm formation and cell-cell aggregation," *Food Microbiol*, vol. 52, pp. 97–105, 2015.

[4] S. Hans, H. Kim, V. Jos, and S. C. J. De Keersmaecker, "*Salmonella* biofilms: An overview on occurrence, structure, regulation and eradication," *Food Research International*, vol. 45, pp. 502–531, 2015.

[5] I-M. Maricarmen, G-L. Melesio, G-M. Pedro, and A-N. María, "Biofilm formation by Staphylococcus aureus and Salmonella spp. Under mono and dual-species conditions and their sensitivity to cetrimonium bromide, peracetic acid and sodium hypochlorite," *Brazilian Journal of Microbiology*, vol. 17, pp. 312–322, 2017.

[6] K. N. C. Maria, B. Elli, J. N. George, and G. Efstathios, "Differential Biofilm Formation and Chemical Disinfection Resistance of Sessile Cells of *Listeria monocytogenes* Strains under Monospecies and Dual-Species (with *Salmonellaenterica*) Conditions," *Applied and Environmental Microbiology*, vol. 78, no. 8, pp. 2586–2595, 2012.

[7] A. C. Rezende, M. C. Igarashi, M. T. Destro, D. G. M. F. Bernadette, and L. Mariza, "Effect of Gamma Radiation on the Reduction of *Salmonella*strains, *Listeria monocytogenes,* and Shiga Toxin-Producing Escherichia coli and Sensory Evaluation of Minimally Processed Spinach (Tetragoniaexpansa)," *Journal of Food Protection®*, vol. 10, pp. 1656–1833, 2014.

[8] D. H. Russell, J. H. Holly, A. Samina, and G. Geffrey, "Increased resistance to multiple antimicrobials and altered resistance gene expression in CMY-2-positive *Salmonella* enterica following a simulated patient treatment with ceftriaxone," *Applied and Environmental Microbiology*, vol. 78, no. 22, pp. 8062–8066, 2012.

[9] N. Grantcharova, V. Peters, C. Monteiro, K. Zakikhany, and U. Römling, "Bistable expression of CsgD in biofilm development of Salmonella enterica serovar typhimurium," *Journal of Bacteriology*, vol. 192, no. 2, pp. 456–466, 2010.

[10] I. Čabarkapa, M. Škrinjar, J. Lević et al., "Biofilm forming ability of salmonella enteritidis in vitro," *Acta Veterinaria-Beograd*, vol. 65, no. 3, pp. 371–389, 2015.

[11] M. A. K. K. P. Perera, "Irradiation as a non-destructive method of food preservation," *Journal of Nutritional Health Sciences*, vol. 1, no. 3, 2017.

[12] K. A. An, Y. Jo, M. S. Arshad, G. R. Kim, C. Jo, and J. H. Kwon, "Assessment of microbial and radioactive contaminations in korean cold duck meats and electron beam application for quality improvement," *Journal of Nutritional Health Sciences*, vol. 37, no. 2, pp. 297–304, 2017.

[13] N. Ben Miloud, A. Elmay, Chatti et al., "Etude de l'effet des radiations gamma sur la viabilité et l'expression différentielle de gènes chez *Salmonella*," *Microbiologie Hygiène Alimentaire*, vol. 23, no. 68, pp. 10–15, 2011.

[14] A. R. Julie, B. Nidhi, Q. Jiang, Z. Weiling, and Cristina M. F., "Effects of ionizing radiation on biological molecules mechanisms of damage and emerging methods of detection," *Antioxid. Redox Signal*, vol. 21, no. 2, pp. 260–292, 2013.

[15] S. Snoussi, A. E. May, L. Coquet, P. Chan, T. Jouenne et al., "Adaptation of Salmonella enterica Hadar under static magnetic field: Effects on outer membrane protein pattern," *Proteome Science*, vol. 10, no. 6, 2012.

[16] W-M. Michal, S. Dana, Z. Yizhou, P. Riky, B. Eddy et al., "Biofilm formation by and multicellular behavior of *escherichia coli* o55:h7, an atypical enteropathogenic strain," *Applied and Environmental Microbiology*, vol. 76, no. 5, pp. 1545–1554, 2010.

[17] D. J. Freeman, F. R. Falkiner, and C. T. Keane, "New method for detecting slime production by coagulase negative staphylococci," *Journal of Clinical Pathology*, vol. 42, no. 8, pp. 872–874, 1989.

[18] T. Møretrø, H. Lene, L. H. Askild, S. S. Maan, R. Knut, and L. Solveig, "Biofilm formation and the presence of the intercellular adhesion locus ica among staphylococci from food and

food processing environments," *Applied and Environmental Microbiology*, vol. 69, no. 9, pp. 5648–5655, 2003.

[19] R. K. Agarwal, S. Singh, K. N. Bhilegaonkar, and V. P. Singh, "Optimization of microtitre plate assay for the testing of biofilm formation ability in different Salmonella serotypes," *International Food Research Journal*, vol. 18, no. 4, pp. 1493–1498, 2011.

[20] C. V. De Oliveira Débora, J. A. Fernandes, R. Kaneno, G. S. Márcia, J. J. P. Araújo et al., "Ability of *Salmonella* spp. to produce biofilm is dependent on temperature and surface material," *Foodborne Pathogens and Disease*, vol. 11, no. 6, pp. 478–483, 2014.

[21] C. Bintao, M. Peter, D. A. Smooker, M. Rouch, and A. Deighton, "Deighton. Selection of suitable reference genes for gene expression studies in *Staphylococcus* capitis during growth under erythromycin stress," *Molecular Genetics and Genomics*, vol. 291, pp. 1795–1811, 2016.

[22] B. R. Anna Carolina, C. I. Maria, T. D. Maria, D. G. M. F. Bernadette, and L. Mariza, "Effect of Gamma Radiation on the Reduction of Salmonella strains, Listeria monocytogenes, and Shiga Toxin–Producing Escherichia coli and Sensory Evaluation of Minimally Processed Spinach (Tetragonia expansa)," *Journal of Food Protection*, vol. 77, no. 10, pp. 1768–1772, 2014.

[23] B. E. Rangaswamy, K. P. Vanitha, and B. S. Hungund, "Microbial Cellulose Production from Bacteria Isolated from Rotten Fruit," *International Journal of Polymer Science*, vol. 2015, Article ID 280784, 8 pages, 2015.

[24] N. Ch. Yassine, M. Sara, R. Christophe, A. Stephane, and H. Julie, "Characterisation of pellicles formed by acinetobacter baumannii at the air-liquid interface," *PLoS ONE*, vol. 9, no. 10, Article ID e111660, 2014.

[25] K. Basar, A. Nefise, and A. Mustafa, "Biofilm-producing abilities of Salmonella strains isolated from Turkey," *Biologia*, vol. 68, no. 1, pp. 1–10, 2013.

[26] B. Ebrahim, H. Azam, M. Reza, A. Seyed-Abdolhamid, and A. Nour, "Biofilm formation in clinical isolates of nosocomial Acinetobacter baumannii and its relationship with multidrug resistance," *Asian Pacific Journal of Tropical Biomedicine*, vol. 6, no. 6, pp. 528–533, 2016.

[27] S. M. Dubravka, Z. P. Bojana, J. V. Maja, L. J. P. Marko, and S. Č. Ivana, "RDAR morpho type- a resting stage of some Enterobacteriaceae," *Food and Feed Research*, vol. 42, no. 1, pp. 43–50, 2015.

[28] K. C. Kalaivani, C. C. Lay, and L. T. Kwai, "Variations in motility and biofilm formation of Salmonella enterica serovar Typhi," *Gut Pathogens*, vol. 6, no. 2, 2014.

[29] T. W. R. Chia, T. A. McMeekin, N. Fegan, and G. A. Dykes, "Significance of the rdar and bdar morphotypes in the hydrophobicity and attachment to abiotic surfaces of *Salmonella* Sofia and other poultry-associated *Salmonella* serovars," *Letters in Applied Microbiology*, vol. 53, no. 5, pp. 581–584, 2011.

[30] G-G. Diego and P. Rafael, "Influence of environmental factors on bacterial biofilm formation in the food industry: a review," *PostDoc Journal*, vol. 3, no. 6, 2015.

[31] O. Kh. Simon, J. Charafeddine, A. Marwan, B. Rabah, and F. Christine, "Effect of incubation duration, growth temperature, and abiotic surface type on cell surface properties, adhesion and pathogenicity of biofilm-detached Staphylococcus aureus cells," *AMB*, vol. 8, no. 9, pp. 3326–3346, 2017.

[32] M. Iñiguez-Moreno, M. Gutiérrez-Lomelí, P. J. Guerrero-Medina, and M. G. Avila-Novoa, "Biofilm formation by Staphylococcus aureus and Salmonella spp. under mono and dual-species conditions and their sensitivity to cetrimonium bromide, peracetic acid and sodium hypochlorite," *Brazilian Journal of Microbiology*, 2017.

[33] A. N. Alonso, M. Montesalvo, A. Rodriguez1, and P. M. Regan, "Effect of 60Co Irradiation on Biofilms Produced by *SalmonellaEnterica*," *Brazilian Journal of Microbiology*, vol. 2, no. 1, pp. 63–69, 2016.

[34] L. Zhen, N. Hua, W. Shuyan, and H. Rui, "CsgD regulatory network in a bacterial trait-altering biofilm formation," *Emerging Microbes and Infections*, vol. 3, 2014.

[35] K. Zakikhany, C. R. Harrington, M. Nimtz, J. C. Hinton, and U. Römling, "Unphosphorylated CsgD controls biofilm formation in Salmonella enterica serovar Typhimurium," *Molecular Microbiology*, vol. 77, no. 3, pp. 771–786, 2010.

[36] F. Ben Abdallah, L. Rihab, S. Khaled, K. Héla, and G. Jawhar, "Detection of cell surface hydrophobicity, biofilm and fimbriae genes in *Salmonella*isolated from Tunisian clinical and Poultry meat," *Iranian Journal of Public Health*, vol. 42, no. 4, pp. 423–431, 2014.