https://doi.org/10.17221/199/2017-CJFS

Effect of Different Conditions on *Listeria monocytogenes* Biofilm Formation and Removal

Pasquale RUSSO[1], Agni HADJILOUKA[2], Luciano BENEDUCE[1], Vittorio CAPOZZI[1], Spiros PARAMITHIOTIS[2], Eleftherios H. DROSINOS[2] and Giuseppe SPANO[1]*

[1]Department of the Sciences of Agriculture, Food and Environment Sciences, University of Foggia, Foggia, Italy; [2]Department of Food Science and Human Nutrition, Agricultural University of Athens, Athens, Greece

**Corresponding author: pasquale.russo@unifg.it*

Abstract

Russo P., Hadjilouka A., Beneduce L., Capozzi V., Paramithiotis S., Drosinos E.H., Spano G. (2018): Effect of different conditions on *Listeria monocytogenes* biofilm formation and removal. Czech J. Food Sci., 36: 208–214.

Listeria monocytogenes poses a major risk for the safety of food products due to the ability to persist in food products and process line surfaces as biofilm. In this work, we investigated the *L. monocytogenes* biofilms in relation to development factors and possible control under different conditions. In particular, the ability of six strains of *L. monocytogenes* from vegetable and animal sources to form biofilms was evaluated on glass or polystyrene substrates under different temperatures (15, 30 and 37°C) and availability of nutrients, by using rich (BHI) or poor (HTM) growth media. Moreover, the effectiveness of three commonly used sanitizers (benzalkonium chloride, sodium hypochlorite and hydrogen peroxide) was compared to eradicate established biofilms. Our results showed that starved conditions, hydrophilic surfaces, and high temperatures increased the *L. monocytogenes* ability to produce biofilms. In general, benzalkonium chloride was the most effective chemical to remove established biofilms.

Keywords: abiotic surfaces; chemical sanitizers; starvation

The foodborne pathogen *Listeria monocytogenes* is the causative agent of listeriosis, a severe disease with high hospitalization and case fatality rates. *L. monocytogenes* can survive and grow in a wide range of adverse environmental conditions typical of the food processing and preservation (Gandhi & Chikindas 2007). A critical point is the risk of *Listeria monocytogenes* persistence in food industry equipment and produce due to its ability to form biofilms (Da Silva & De Martinis 2013; Ferreira *et al.* 2014). Microbial films can occur on different industrial working surfaces including floor drains, conveyer belts, rubber, plastic, glass, stainless steel, and other food-contact materials (Shi & Zhu 2009; Carpentier & Cerf 2011; Srey *et al.* 2013). Moreover, biofilms confer protection to bacterial cells and decrease the efficiency of cleaning and disinfection procedures, increasing the risk of cross contamination during processing of the raw material (Møretrø & Langsrud 2004; Van Houdt & Michiels 2010). In particular, *L. monocytogenes* poses a serious risk to the human health in minimally processed food, such as fresh-cut fruits and vegetables, since the control of the microbial load is mainly based on the respect of the cold chain (Russo *et al.* 2014). In recent years, contaminated cantaloupes were the causative agents of the deadliest foodborne outbreak in the United States since the 1920s (CDC 2011). Fatal events like

Supported by European Union's Seventh Framework Programme for research, technological development, and demonstration under Grant agreement, Projects No. 289719 and PONREC2007–2013.

https://doi.org/10.17221/199/2017-CJFS

this outbreak underscore the importance to increase the knowledge of *L. monocytogenes* biofilm formation and to propose efficient strategies to prevent their development. In the last years, several authors investigated the relationships between biofilms and the main factors involved in their formation, but the conclusions were often divergent (Chavant *et al.* 2002; Di Bonaventura *et al.* 2008; Nilsson *et al.* 2011; Choi *et al.* 2013; Kadam *et al.* 2013; Bonsaglia *et al.* 2014). Therefore, it is evident that this matter needs further insights. Indeed, as recently suggested by Nowak *et al.* (2015), biofilm formation seems not be narrowed down to one factor but rather dependent on multiple factors, with temperature and nutrient availability being the most important.

The most commonly used chemical agents for sanitation procedures are oxidizing agents with a broad antimicrobial spectrum: hypochlorous acid, chlorine, iodine, ozone, hydrogen peroxide, peroxyacetic acid, quaternary ammonium chloride and anionic acids (Simões *et al.* 2010; Da Silva & De Martinis 2013). The effectiveness of these sanitizers for *L. monocytogenes* biofilm eradication was studied and compared at different experimental conditions in order to establish the best treatment for specific purposes (Vaid *et al.* 2010; Belessi *et al.* 2011). In alternative to conventional chemical-based approaches, physical methods or 'green-strategies' have also been proposed as innovative techniques to control the biofilm formation (Simões *et al.* 2010). Nonetheless, the application of chemicals still remains the only step employed in the food industry to reduce the bacterial load.

In this work, we investigated, as a preliminary step how the production of *L. monocytogenes* biofilms is affected by different surfaces, growth temperatures and nutrient availability. In addition, three different sanitizers usually employed in the food industry were analysed for their effectiveness to remove the established biofilm.

MATERIAL AND METHODS

Microbial strains and growth conditions. Five *L. monocytogenes* strains (serotype 4b) of strawberry origin (namely, LQC 15260, 15261, 15262, 15258, and 15259) were provided by the culture collection of the Laboratory of Food Quality Control and Hygiene, Agricultural University of Athens (Greece). The reference strain *L. monocytogenes* CECT 4031T (serotype 1/2a) was purchased at the Spanish Type Culture Collection (CECT, Spain). Strains were routinely grown on Brain Heart Infusion (BHI) (Oxoid, UK) at 37°C for 24 hours.

Biofilm determination. *L. monocytogenes* strains were propagated from glycerol stock in BHI by incubating at 37°C until mid-exponential phase. Cultures were diluted (1 : 100, v/v) in BHI or Hsiang-Ning Tsai medium (HTM) (Tsai & Hodgson 2003) broth, and distributed in 96-well polystyrene microtitre plates (each well containing 200 µl) or glass tubes (5 ml). Plates and tubes were incubated at 15, 30 and 37°C for 72 hours. Then, biofilm production was measured by using crystal violet staining according to Djordjevic *et al.* (2002). Briefly, wells and tubes were gently washed three times with sterile water to remove non-adherent cells. Subsequently, the biofilm was stained with 0.05% (w/v) crystal violet for 40 min, followed by washing three times with sterile water to remove the excess of crystal violet. After drying, crystal violet was dissolved in 96% ethanol and absorbance was measured at 590 nm. Absorbance was corrected for the mean absorbance value of the blank. Experiments were performed in triplicate, and each assay was the average of five replicates.

Biofilm removal by sanitizing treatments. Solutions of sodium hypochlorite (200 ppm, v/v), hydrogen peroxide (2%, v/v), and benzalkonium chloride (200 ppm, w/v) were tested as sanitizers for their ability to remove the established biofilm. *L. monocytogenes* strains LQC 15260, 15262, 15258, and CECT 4031T were incubated in glass tubes at 37°C for 72 h by using HTM as media to promote the biofilm formation. After the removal of the media, 5 ml of each biocide solution were added and incubated for 10 min at room temperature. The control samples were treated with sterile water. Then, sanitizers were eliminated and the biofilm quantification was spectrophotometrically determined as reported above. Experiments were performed in triplicate, and each assay was the average of five replicates.

Statistical analysis. Data were subjected to one-way analysis of variance (ANOVA). Pairwise comparison of treatment means was achieved by Tukey's procedure with a significance level of $P < 0.05$, using the statistical software Past 3.0.

RESULTS AND DISCUSSION

L. monocytogenes biofilm formation. Five strains of *L. monocytogenes* serotype 4b isolated from straw-

https://doi.org/10.17221/199/2017-CJFS

berries and one strain of *L. monocytogenes* serotype 1/2a of animal origin were investigated for their ability to produce biofilms on glass and polystyrene surfaces, at different temperatures (15, 30, and 37°C) by growing the pathogen on rich (BHI) or poor (HTM) culture media.

Biofilm development was quantified by staining with crystal violet as previously reported (Djordjevic *et al.* 2002), and *L. monocytogenes* strains were classified into four groups according to the following scale: no/low ($OD_{590} < 0.2$), moderate ($0.2 < OD_{590} < 0.4$), high ($0.6 < OD_{590} < 1$), and very high ($OD_{590} > 1$) biofilm producers. As shown in Figure 1, the biofilm formation was clearly higher in glass tubes than in microtitre plates at all experimental conditions. In particular, no or low biofilm production ($OD_{590} < 0.2$) was detected for all strains on plastic support, except for the biofilm produced by the strains LQC 15262 and 15258 at 37°C in the poor medium HTM (Figure 1). In contrast, when glass was used as support, no or low biofilm amounts were found only at 15°C by using BHI as a medium (Figure 1).

Interestingly, the highest biofilm production ($OD_{590} > 1$) was revealed from the strain LQC 15258 in glass at 30°C when grown in HTM, which was about twofold more than the biofilm produced at 15 and 37°C at the same experimental conditions. However, a general increase in the biofilm formation was observed depending on the temperature. Indeed, at 15°C no or low biofilm amounts were formed except for strains incubated on glass and HTM. In contrast, the strains LQC 15258 and 15260 were able to produce high levels of biofilm in glass at 30 and 37°C ($0.6 < OD_{590} < 1$), when grown in BHI. Under this condition, the remaining strains always enhanced their biofilm producing ability from low at 30°C ($OD_{590} < 0.2$) to moderate at 37°C ($0.2 < OD_{590} < 0.4$). A similar pattern was observed for the strains LQC 15258 and 15262 in HTM and polypropylene support (Figure 1).

Moreover, we found that the biofilm production in the rich medium BHI was always lower than in HTM, and the effect of the limited availability of nutrients seems to be more evident at low temperatures than at 37°C (Figure 1). Finally, *L. monocytogenes* CECT 4031T was a weaker biofilm producer than the other strains analysed at all the experimental conditions (Figure 1).

Eradication of established biofilm. In a subsequent step the effectiveness of three disinfectants to remove established biofilms was analysed, starting from the higher producer strains, namely LQC 15260, 15262, and 15258, and from the lower producer strain CECT 4031T. According to previous works (Ibusquiza *et al.* 2011; Henriques & Fraqueza 2017), solutions of sodium hypochlorite, hydrogen peroxide, and benzalkonium chloride were analysed for their ability to remove the biofilm formed on glass after 72 h of incubation at 37°C in HTM. These conditions were selected since they seem to promote the biofilm production. As shown in Figure 2, chemical biocides were able to remove *L. monocytogenes* biofilms at different levels. In general, a biofilm reduction of about 60% was observed for the lower producer strain CECT 4031T, independently of the sanitizer applied.

In contrast, remarkable differences were observed in the ability of the three sanitizers to remove the biofilm formed by the higher producer *L. monocytogenes* strains. In general, benzalkonium chloride

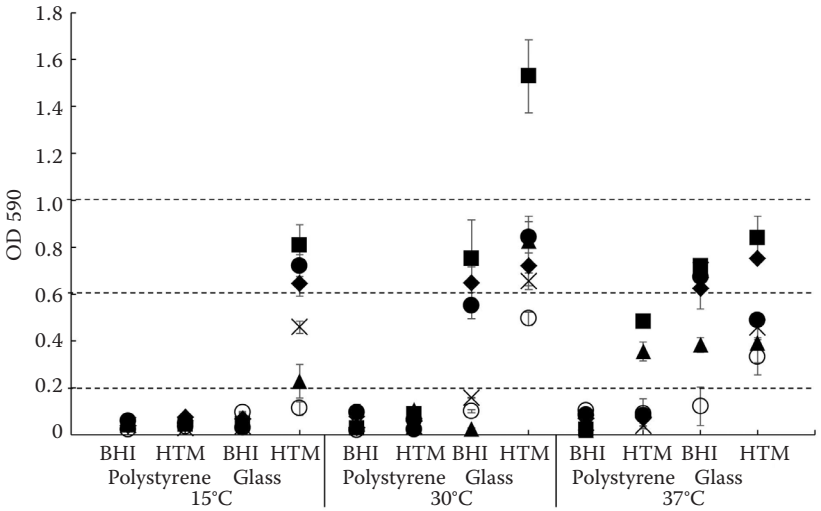

Figure 1. Biofilm formed by the strains LQC 15260 (black diamond), 15261 (black circle), 15262 (black triangle), 15258 (black square), 15259 (asterisk), and CECT 4031T (white circle) after 72 h of incubation at 15, 30, and 37°C, on polystyrene or glass, when grown in BHI or HTM

Dashed lines indicate the range to discriminate between no/low, moderate, high, and very high biofilm producers; experiments were performed in triplicate and the standard deviations are indicated by bars; results are the average of three replicates for each strain

https://doi.org/10.17221/199/2017-CJFS

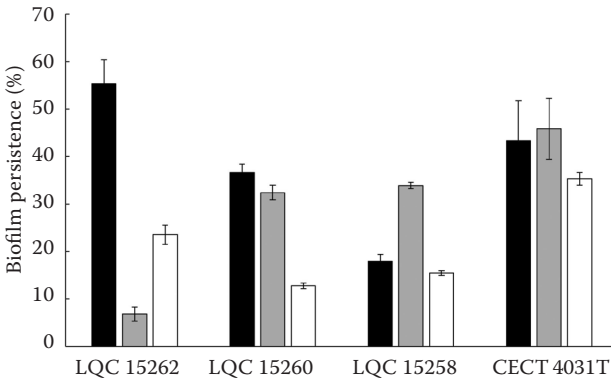

Figure 2. Persistence of biofilms formed by the *L. monocytogenes* strains LQC 15262, 15260, 15258, and CECT 4031T in glass tubes after 72 h at 37°C, and treated with sodium hypochlorite (black bars), hydrogen peroxide (grey bars), and benzalkonium chloride (white bars)

Experiments were performed in triplicate, and the standard deviations are indicated by bars; results are the average of three replicates for each strain

was the most effective chemical, since after its application a reduction of about 88, 85, and 73% was detected for the biofilm formed by the strains LQC 15260, 15258, and 15262, respectively. Interestingly, hydrogen peroxide was able to remove almost completely the biofilm formed by the strain LQC 15262, while for the other strains a reduction of about 70% was observed. In contrast, the biofilm reduction detected after the application of a sodium hypochlorite solution was of 55, 36, and 18% for the strains LQC 15262, 15260, and 15258, respectively.

Biofilm formation by foodborne pathogenic bacteria, such as *L. monocytogenes*, is a critical issue for food safety. Although in the last years several studies have focused on this matter, the knowledge of the conditions promoting the biofilm establishment and possible strategies to facilitate biofilm removal are yet unclear. Therefore, in the present work, six strains of *L. monocytogenes* were evaluated in order to improve the knowledge of the biofilm development under different conditions. In particular, the ability of *L. monocytogenes* (serotype 4b and 1/2a) of vegetable and animal origin to form biofilm was evaluated by comparing the concomitant effect of material surface, temperature, and nutrient availability. With this aim, two different supports commonly used in the food industry, i.e. glass and polystyrene, were selected as representatives of hydrophilic and hydrophobic substrates, respectively. In addition, the ability to form biofilm was tested at a low temperature (15°C), at a mild temperature (30°C) and at the optimum growth temperature for *L. monocytogenes* (37°C). Finally, the biofilm production was investigated under different conditions of nutrient availability to *L. monocytogenes* growing on rich (BHI) or poor (HTM) culture media.

In this work, we generally observed a higher biofilm production on glass than on polystyrene supports, which could suggest a presumptive scarce ability of *L. monocytogenes* to colonize hydrophobic surfaces. In accordance with these findings, faster and higher biofilm formation on hydrophilic rather than on plastic substratum has been reported (Chavant *et al.* 2002). Recently, Bonsaglia *et al.* (2014) found that all tested *L. monocytogenes* strains were able to form biofilm on glass and stainless steel, while only 28% of them retained this feature on polystyrene. Similarly, Di Bonaventura *et al.* (2008) detected a significantly higher biofilm formation on glass than on polystyrene analysing *L. monocytogenes* strains of food and environmental origin. Nonetheless, contrasting results showing a better development of *L. monocytogenes* biofilm on plastic surfaces have also been reported (Djordjevic *et al.* 2002; Stepanović *et al.* 2004; Takahashi *et al.* 2010).

It has been reported that temperature plays an important role for the expression of many virulence and colonization genes in *L. monocytogenes*, sometimes resulting in cell surface structure changes that could affect the bacterial attachment (Liu *et al.* 2002). Furthermore, temperature may influence the cell wall composition modifying its electrical properties, and encouraging the formation of fimbriae, flagella and lipopolysaccharide actively involved in the adhesion to abiotic surfaces (Chavant *et al.* 2002). In the present study, a positive relationship between temperature and biofilm formation was revealed, accordingly to what was previously reported (Nilsson *et al.* 2011; Combrouse *et al.* 2013; Kadam *et al.* 2013). In contrast, Norwood and Gilmour (2001) observed a higher adhesion of *L. monocytogenes* to stainless steel at 18°C, suggesting that high biofilm amounts were depending on the optimal temperature for flagellin expression. However, other authors did not find a clear correlation between the ability of *L. monocytogenes* to produce biofilm and the incubation temperature (Moltz & Martin 2005; Di Bonaventura *et al.* 2008; Bonsaglia *et al.* 2014).

In our work, we found that starvation conditions could promote the biofilm production by *L. monocytogenes*. According to this finding, the growth of *L. monocytogenes* in typical rich media appeared

https://doi.org/10.17221/199/2017-CJFS

not to have the best potential to switch cells from planktonic to sessile state, while growth in poor synthetic media enhanced attachment and biofilm formation (Chavant *et al.* 2002; Djordjevic *et al.* 2002; Zhou *et al.* 2012; Combrouse *et al.* 2013). In agreement, the highest biofilm production was observed in the chemically defined poor medium HTM when compared with three different nutrient-rich media (Kadam *et al.* 2013). Likewise, the biofilm formation increased when *L. monocytogenes* was grown in minimal media rather than in a complex broth (Zhou *et al.* 2012; Combrouse *et al.* 2013). However, contrasting results may indicate that the ability to produce biofilm under different nutrient conditions is a strain-dependent feature of *L. monocytogenes* (Moltz & Martin 2005; Folsom *et al.* 2006). In contrast, Choi *et al.* (2013) observed that the biofilm formed in TSB was higher than in diluted TSB. Similarly, the attachment of *L. monocytogenes* to a stainless steel surface increased when cultivated in BHI rather than in starved conditions (Mai & Conner 2007).

Among all the analysed strains, we found that *L. monocytogenes* CECT 4031T was a weaker biofilm producer. However, a higher number of strains should be analysed in further investigations to clearly establish if vegetable isolates or serovar 4b are the best biofilm producers. Indeed, it was previously reported that the origin of the isolate did not significantly affect the level of biofilm formation (Kadam *et al.* 2013). However, Folsom *et al.* (2006) found that serotype 4b strains produced more biofilm than serotype 1/2a, proposing a different regulation of the biofilm phenotype. Inter-strain variations seem to be associated with the serotype 1/2c that was found to adhere significantly more than strains of serotypes 1/2a and 4b (Norwood & Gilmour 1999; Di Bonaventura *et al.* 2008). In contrast, Nilsson *et al.* (2011) detected higher biofilm production by serotype 1/2a strains, while serotype 1/2b and 1/2a strains were able to produce higher biofilm than the serotype 4b strains at 37°C in BHI broth (Kadam *et al.* 2013). However, a recent study showed that different strains of *L. monocytogenes* form the biofilm of different intensities which did not completely correlate with their serotype (Doijad *et al.* 2015).

In the last years, several studies tested the application of different sanitizing compounds for their ability to remove *L. monocytogenes* biofilms from the food premises (Ibusquiza *et al.* 2011; Torlak & Sert 2013; Ban & Kang 2016). According to Purkrtová *et al.* (2010) we found the high effectiveness of benzalkonium chloride to remove established biofilms. However, its use in the food industry might be limited by the potential development of resistant isolates. Indeed, *L. monocytogenes* strains harbouring the benzalkonium chloride resistance cassette *bcrABC* could be involved in the horizontal widespread dissemination of *bcrABC* across *L. monocytogenes* genomes (Dutta *et al.* 2013). Interestingly, a high variability was detected in the ability of sodium hypochlorite and hydrogen peroxide to remove *L. monocytogenes* biofilms. We hypothesized that different oxidizing capacity could be related to the high complexity of the biofilm structures, depending on the degree of its maturation or on strain depending traits of the biofilm matrix composition. According to these suggestions, structural features such as maximum thickness, biovolume, areal porosity and maximum diffusion distance revealed differences in the biofilm formation of different *L. monocytogenes* strains (Guilbaud *et al.* 2015; Mosquera-Fernández *et al.* 2016).

Finally, it is worth emphasizing that, although significant reductions were observed, none of the tested biocides was able to completely remove the established biofilm in the experimental conditions. This finding is particularly interesting since an exposure to subminimal inhibitory concentrations of the disinfectant seems to encourage the proliferation of resilient populations of *L. monocytogenes* (Ortiz *et al.* 2014).

CONCLUSIONS

In conclusion, in the present work, we reported that hydrophilic surfaces and higher temperatures seem to increase the ability of *L. monocytogenes* strains to form biofilms. In the food industry this risk could be reduced by an accurate control of the cold chain in the working environments. Nonetheless, poor nutritional milieus could enhance the biofilm production also at the lower temperatures found in the food processing plants, where the release of cellular nutrients and the occurrence of food debris might create suitable conditions for biofilm establishment and proliferation. Furthermore, although the application of commonly used chemical sanitizers was unable to completely eradicate mature *L. monocytogenes* biofilms, benzalkonium chloride seems to be the most effective to remove established biofilms.

https://doi.org/10.17221/199/2017-CJFS

References

Ban G.H., Kang D.H. (2016): Effect of sanitizer combined with steam heating on the inactivation of foodborne pathogens in a biofilm on stainless steel. Food Microbiology, 55: 47–54.

Belessi C.E., Gounadaki A.S., Psomas A.N., Skandamis P.N. (2011): Efficiency of different sanitation methods on *Listeria monocytogenes* biofilms formed under various environmental conditions. International Journal of Food Microbiology, 145: 46–52.

Bonsaglia E.C.R., Silva N.C.C., Fernades Júnior A., Araújo Júnior J.P., Tsunemi M.H., Rall V.L.M. (2014): Production of biofilm by *Listeria monocytogenes* in different materials and temperatures. Food Control, 35: 386–391.

Carpentier B., Cerf O. (2011): Review – Persistence of *Listeria monocytogenes* in food industry equipment and premises. International Journal of Food Microbiology, 145: 1–8.

CDC (2011): Multistate outbreak of listeriosis associated with Jensen Farms cantaloupe – United States, August –September 2011. Morbidity and Mortality Weekly Report, 60: 1357–1358.

Chavant P., Martinie B., Meylheuc T., Bellon-Fontaine M.N., Hebraud M. (2002): *Listeria monocytogenes* LO28: surface physicochemical properties and ability to form biofilms at different temperatures and growth phases. Applied and Environmental Microbiology, 68: 728–737.

Choi N.Y., Kim B.R., Bae Y.M., Lee S.Y. (2013): Biofilm formation, attachment, and cell hydrophobicity of foodborne pathogens under varied environmental conditions. Journal of the Korean Society for Applied Biological Chemistry, 56: 207–220.

Combrouse T., Sadovskaya I., Faille C., Kol O., Guérardel Y., Midelet-Bourdin G. (2013): Quantification of the extracellular matrix of the *Listeria monocytogenes* biofilms of different phylogenic lineages with optimization of culture conditions. Journal of Applied Microbiology, 114: 1120–1131.

da Silva E.P., de Martinis E.C. (2013): Current knowledge and perspectives on biofilm formation: the case of *Listeria monocytogenes*. Applied Microbiology and Biotechnology, 97: 957–968.

di Bonaventura G., Piccolomini R., Paludi D., D'Orio V., Vergara A., Conter M., Ianieri A. (2008): Influence of temperature on biofilm formation by *Listeria monocytogenes* on various food-contact surfaces: relationship with motility and cell surface hydrophobicity. Journal of Applied Microbiology, 104: 1552–1561.

Djordjevic D., Wiedmann M., McLandsborough L.A. (2002): Microtiter plate assay for assessment of *Listeria monocytogenes* biofilm formation. Applied and Environmental Microbiology, 68: 2950–2958.

Doijad S.P., Barbuddhe S.B., Garg S., Poharkar K.V., Kalorey D.R., Kurkure N.V., Rawool D.B., Chakraborty T. (2015): Biofilm-forming abilities of *Listeria monocytogenes* serotypes isolated from different sources. PLoS ONE: E0137046. doi 10.1371/journal.pone.0137046

Dutta V., Elhanaf D., Kathariou S. (2013): Conservation and distribution of the benzalkonium chloride resistance cassette *bcrABC* in *Listeria monocytogenes*. Applied and Environmental Microbiology, 79: 6067–6074.

Ferreira V., Wiedmann M., Teixeira P., Stasiewicz M.J. (2014): *Listeria monocytogenes* persistence in food-associated environments: epidemiology, strain characteristics, and implications for public health. Journal of Food Protection, 77: 150–170.

Folsom J.P., Siragusa G.R., Frank J.F. (2006): Formation of biofilm at different nutrient levels by various genotypes of *Listeria monocytogenes*. Journal of Food Protection, 69: 826–834.

Gandhi M., Chikindas M.L. (2007): *Listeria*: A foodborne pathogen that knows how to survive. International Journal of Food Microbiology, 113: 1–15.

Guilbaud M., Piveteau P., Desvaux M., Brisse S., Briandet R. (2015): Exploring the diversity of *Listeria monocytogenes* biofilm architecture by high-throughput confocal laser scanning microscopy and the predominance of the honeycomb-like morphotype. Applied and Environmental Microbiology, 81: 1813–1819.

Henriques A.R., Fraqueza M.J. (2017): Biofilm-forming ability and biocide susceptibility of *Listeria monocytogenes* strains isolated from the ready-to-eat meat-based food products food chain. LWT-Food Science and Technology, 81: 180–187.

Ibusquiza P.S., Herrera J.J.R., Cabo M.L. (2011): Resistance to benzalkonium chloride, peracetic acid and nisin during formation of mature biofilms by *Listeria monocytogenes*. Food Microbiology, 28: 418–425.

Kadam S.R., den Besten H.M., van der Veen S., Zwietering M.H., Moezelaar R., Abee T. (2013): Diversity assessment of *Listeria monocytogenes* biofilm formation: impact of growth condition, serotype and strain origin. International Journal of Food Microbiology, 165: 259–264.

Liu S., Graham J.E., Bigelow L., Morse P.D., Wilkinson B.J. (2002): Identification of *Listeria monocytogenes* genes expressed in response to growth at low temperature. Applied and Environmental Microbiology, 68: 1697–1705.

Mai T.L., Conner D.E. (2007): Effect of temperature and growth media on the attachment of *Listeria monocytogenes* to stainless steel. International Journal of Food Microbiology, 120: 282–286.

https://doi.org/10.17221/199/2017-CJFS

Moltz A.G., Martin S.E. (2005): Formation of biofilms by *Listeria monocytogenes* under various growth conditions. Journal of Food Protection, 68: 92–97.

Møretrø T., Langsrud S. (2004): *Listeria monocytogenes*: biofilm formation and persistence in food-processing environments. Biofilms, 1: 107–121.

Mosquera-Fernández M., Sanchez-Vizuete P., Briandet R., Cabo M.L., Balsa-Canto E. (2016): Quantitative image analysis to characterize the dynamics of *Listeria monocytogenes* biofilms. International Journal of Food Microbiology, 236: 130–137.

Nilsson R.E., Ross T., Bowman J.P. (2011): Variability in biofilm production by *Listeria monocytogenes* correlated to strain origin and growth conditions. International Journal of Food Microbiology, 150: 14–24.

Norwood D.E., Gilmour A. (1999): Adherence of *Listeria monocytogenes* strains to stainless steel coupons. Journal of Applied Microbiology, 86: 576–582.

Norwood D.E., Gilmour A. (2001): The differential adherence capabilities of two *Listeria monocytogenes* strains in monoculture and multispecies biofilms as a function of temperature. Letters in Applied Microbiology, 33: 320–324.

Nowak J., Cruz C.D., Palmer J., Fletcher G.C., Flint S. (2015): Biofilm formation of the *L. monocytogenes* strain 15G01 is influenced by changes in environmental conditions. Journal of Microbiological Methods, 119: 189–195.

Ortiz S., López V., Martínez-Suárez J.V. (2014): The influence of subminimal inhibitory concentrations of benzalkonium chloride on biofilm formation by *Listeria monocytogene*s. International Journal of Food Microbiology, 189: 106–112.

Purkrtová S., Turonová H., Pilchová T., Demnerová K., Pazlarová J. (2010): Resistance of *Listeria monocytogenes* biofilms to disinfectants. Czech Journal of Food Sciences, 28: 326–332.

Russo P., Botticella G., Capozzi V., Massa S., Spano G., Beneduce L. (2014): A fast, reliable, and sensitive method for detection and quantification of *Listeria monocytogenes* and *Escherichia coli* O157:H7 in ready-to-eat fresh-cut products by MPN-qPCR. BioMed Research International, Article ID 608296. doi 10.1155/2014/608296

Shi X., Zhu X. (2009): Biofilm formation and food safety in food industries. Trends in Food Science and Technology, 20: 407–413.

Simões M., Simões L.C., Vieira M.J. (2010): A review of current and emergent biofilm control strategies. LWT-Food Science and Technology, 43: 573–583.

Srey S., Jahid I.K., Ha S.D. (2013): Biofilm formation in food industries: A food safety concern. Food Control, 31: 572–585.

Stepanović S., Vuković D., Hola V., Di Bonaventura G., Djukić S., Cirković I., Ruzicka F. (2007): Quantification of biofilm in microtiter plates: overview of testing conditions and practical recommendations for assessment of biofilm production by staphylococci. APMIS, 115: 891–899.

Takahashi H., Suda T., Tanaka Y., Kimura B. (2010): Cellular hydrophobicity of *Listeria monocytogenes* involves initial attachment and biofilm formation on the surface of polyvinyl chloride. Letters in Applied Microbiology, 50: 618–625.

Torlak E., Sert D. (2013): Combined effect of benzalkonium chloride and ultrasound against *Listeria monocytogenes* biofilm on plastic surface. Letters in Applied Microbiology, 57: 220–226.

Tsai H.N., Hodgson D.A. (2003): Development of a synthetic minimal medium for *Listeria monocytogenes*. Applied and Environmental Microbiology, 69: 6943–6945.

Vaid R., Linton R.H., Morgan M.T. (2010): Comparison of inactivation of *Listeria monocytogenes* within a biofilm matrix using chlorine dioxide gas, aqueous chlorine dioxide and sodium hypochlorite treatments. Food Microbiology, 27: 979–984.

van Houdt R., Michiels C.W. (2010): Biofilm formation and the food industry, a focus on the bacterial outer surface. Journal of Applied Microbiology, 109: 1117–1131.

Zhou Q., Feng X., Zhang Q., Feng F., Yin X., Shang J., Qu H., Luo Q. (2012): Carbon catabolite control is important for *Listeria monocytogenes* biofilm formation in response to nutrient availability. Current Microbiology, 65: 35–43.

Received: 2017–05–30
Accepted after corrections: 2018–02–16
Published online: 2018–06–04