® Postepy Hig Med Dosw (online), 2019; 73: 937–943
e-ISSN 1732-2693

www.phmd.pl
Review

Received: 08.01.2019
Accepted: 18.04.2019
Published: 31.12.2019

Salmonella biofilm development: Structure and significance

Rozwój biofilmu *Salmonella* spp. – budowa i znaczenie

Katarzyna Ćwiek[1], Gabriela Bugla-Płoskońska[2], Alina Wieliczko[1]

[1] Department of Epizootiology with Clinic of Birds and Exotic Animals, Wrocław University of Environmental and Life Sciences
[2] Department of Microbiology, Institute of Genetics and Microbiology, University of Wrocław

Summary

Salmonella spp. is capable of adhering and forming a biofilm on materials of different kinds during their life cycle, contaminating the food chain, thus representing a potential danger for consumers. This review discusses the ability of *Salmonella* to form biofilm as the main obstacle to reducing the prevalence of these pathogens in food production. The components of *Salmonella* biofilm, such as cellulose, curli fimbriae, outer membrane proteins (OMPs) and their molecular bases are described, as well as various *Salmonella* morphotypes (rdar, bdar, pdar and saw). OMPs play very important roles in the cells of *Salmonella* strains, because they are at the interface between the pathogenic cells and the host tissue and they can contribute to adherence, colonization, virulence and biofilm formation. Furthermore, the importance of *quorum sensing* is discussed as a crucial factor regulating the properties of biofilm formation and pathogenicity. To further illustrate that biofilm formation is a mechanism used by *Salmonella* to adapt to various environments, the resistance of *Salmonella* biofilms against different stress factors including antimicrobials (disinfectants, antibiotics and plant extracts) is described.

Keywords: ***Salmonella*** **• biofilm • foodborne pathogen • cellulose • curli fimbriae**

GICID 01.3001.0013.7866
DOI: 10.5604/01.3001.0013.7866
Word count: 5358
Tables: –
Figures: –
References: 72

Author's address: Prof. dr hab. Alina Wieliczko: Department of Epizootiology with Clinic of Birds and Exotic Animals, Wrocław University of Environmental and Life Sciences, Pl. Grunwaldzki 45, 50-366 Wrocław, Poland, e-mail: alina.wieliczko@upwr.edu.pl

INTRODUCTION

According to the newest European Food Safety Authority (EFSA) report, *Salmonella* spp. is still the second highest zoonoses source in the European Union. In 2016, *Salmonella enterica* was responsible for 94.530 confirmed cases of salmonellosis in humans and 128 deaths in the EU. The food-borne pathogen *Salmonella* is a well-known example of bacteria responsible for human and animal infections. The main sources of this pathogen are poultry products, such as chicken meat, eggs and egg products [14]. Infection (salmonellosis) caused by certain members of the genus are a serious epidemiological and economic problem worldwide. Host-to-host transmission in most *Salmonella* serovars occurs generally via the fecal–oral route. Human salmonellosis is an infectious disease occurring in various clinical forms and levels of severity (most often gastroenteritis), usually self-limiting. In poultry, the course of salmonellosis is often asymptomatic and a carrier state may occur, representing a zoonotic threat. Furthermore, some infected individuals, both cattle, swine, poultry and humans, can become carriers and, as a result, can excrete *Salmonella* in their feces at length, and thereby they can function as a reservoir for this pathogen. These infected individuals are crucial items for zoonotic control because they represent a significant way to transmit disease [57, 68]. Moreover, the carrier state in livestock can increase the probability that *Salmonella* survives in a farm environment, in spite of adverse and stressful environmental conditions, and consequently lead to the formation of biofilm outside the host on abiotic surfaces and possible contamination of food products [20].

The barrier to the disadvantageous environmental conditions, which hinders the eradication of microorganisms, is a biofilm – a complex biological structure consisting of many bacterial cells surrounded by layers of substances produced by them. In environmental settings, biofilms are a common element in the life of microorganisms [27]. *Salmonella enterica*, as well as other species from the *Enterobacteriaceae* family, is capable of adhering and forming biofilm on materials of different kinds during their life cycle [29, 45]. This ability of non-enterica subspecies (*Salmonella enterica* subsp. *arizonae/diarizonae/houtenae/salamae*) is more poorly examined but is described and compared briefly below. No studies have been found relevant to the species *Salmonella bongori* and its ability to produce biofilm thus our review only applies to *Salmonella enterica*.

Although the main sources of *Salmonella* are poultry products, fresh products, such as fruits and vegetables, are increasingly contaminated with *Salmonella*, even those cultivated by pumped irrigation water. The consequences of consuming such products without heat treatment are a serious public health problem in view of salmonellosis outbreaks [35, 64]. Next to the global problem of increasing antimicrobial resistance, announced by the World Health Organisation (WHO), the ability of bacteria to form biofilm may cause therapeutic problems in human and veterinary medicine. The literature provides a number of publications [32, 41, 42, 59] claiming that biofilms, including those formed by *Salmonella enterica*, are more resistant to antimicrobial factors than planktonic forms. This aspect is discussed in a later part of the review.

SALMONELLA BIOFILM TYPES

The reasons that bacteria aggregate together are still not fully understood. Xavier et al. [69] suggest that it could be the result of cells competing with each other for access to nutrients and oxygen. The formation of biofilms may enable the survival of *Salmonella*, allowing it to resit such stresses as antimicrobials, extreme temperatures, low-nutrition conditions, acidic pH and different atmospheres [25, 56].

There are a few *Salmonella* biofilm types, such as pellicles, that appear at the air-liquid interface as a film of cells in standing liquid cultures [53]. The accumulated multicellular aggregate in the bottom of the flask is a different type of *Salmonella* biofilm. In this model, described in detail by MacKenzie et al. [31], planktonic cells are suspended in the growth media in relatively stable proportions to multicellular aggregates. The well-known *Salmonella* biofilm phenotype, called rdar on account of the colony appearance on Congo Red agar plates, is described in detail below, as well as a less frequent *Salmonella* morphotype.

BIOFILM FORMING ABILITY WITHIN THE ENTIRE *SALMONELLA* GENUS

Salmonella strains, intensively producing biofilm, can persist in the food chain and consequently contaminate food products, resulting in a negative impact on public health. A well-defined and studied serovar of subspecies enterica is *Salmonella* Typhimurium, while *S.* Enteritidis as a zoonotic and food-borne serovar like *S.* Typhimurium has been described in a few studies [25, 33, 42, 54]. According to the EFSA report, *S.* Enteritidis and *S.* Typhimurium are the most significant serovars with regards to foodborne outbreaks [14]. Lamas et al. [24] described the capability of different subspecies forming biofilm: *arizonae, diarizonae* and *salamae* on polystyrene surfaces. However, some strains of the subspecies *salamae* have shown more biofilm formation ability than other subspecies. Moreover, in that study all the examined strains of the subspecies *salamae* produce cellulose and curli fimbriae, which are crucial extracellular components and are described in detail below. The production of both of these components differs among *S. enetrica* as well as other subspecies, with the exception of subspecies *arizonae* [65].

OCCURRENCE OF *SALMONELLA* BIOFILM

Numerous studies have reported that *Salmonella enterica* serovars are able to adhere and form biofilm on plastic, glass, even on stainless steel [19, 21, 67]. These materials are commonly used in kitchens and toilets but, above

all, in slaughter houses, farms and the the food industry. Taking into account the ability of *Salmonella* to survive on abiotic surfaces, *Salmonella* can represent a potential danger for consumers by contaminating food products. A number of publications [44, 50, 63] describe *Salmonella* strains being associated with fruit, nut or vegetable contamination outbreaks. Yaron and Römling [70] noticed that *Salmonella* is able to form biofilm on lettuce, tomatoes parsley and cucumber. It should be a major issue for human and animal health and quide the importance of adequate hygiene and disinfection methods in food production to control and prevent the presence of food-borne pathogen biofilms.

Many studies [25, 30, 33, 43, 60] used polystyrene and stainless steel to perform *Salmonella* biofilm assays. Most of them have shown that *Salmonella* strains are able to form biofilm on polystyrene surfaces commonly used in meat or fruit packing as well as in water supplies or feeding stations in poultry farms.

Stepanovic et al. [58] examined more than a hundred *Salmonella enterica* strains for their ability to produce biofilm in both nutrient-rich and nutrient-poor media. It turns out that *Salmonella* produce more biofilm in media lacking in nutritional ingredients. This finding shows that the requirements of this species are not high and the pathogen can survive even in an adverse environment such as the relatively clean surfaces of the food industry or home kitchens. Paz-Mendez et. al [43], unlike other researchers, used food residues, such as tomato and chicken meat juices as well as milk, instead of common growth media, to evaluate how they impact the biofilm-forming ability of *Salmonella enterica* strains. Moreover, these authors used a biofilm assay to test different conditions that the strains can encounter in each step of the food chain, and two types of surfaces (polystyrene and stainless steel). *Salmonella* strains were able to produce biofilm with chicken meat juice growth medium under all conditions. This discovery proves that meat juice can be a nutrient source allowing *Salmonella* to form biofilm during food processing.

The significant ability of *Salmonella* to form microcolonies and even mature biofilms on biotic surfaces such as epithelial cells is well described [7, 28] and is a concern in both veterinary and human medicine. The ability to grow biofilms on chicken intestinal tissue, the Hep-2 model system, was described by Ledeboer et al. [28]. Due to the fact that *Salmonella* is an important member of the intestinal flora of turkeys and chickens, this research can be useful in biofilm formation studies. Understanding the interactions and genetics factors responsible for *Salmonella* biofilm production may facilitate the development of methods to modify and control *Salmonella* colonization as well as the transmission this food-borne pathogen makes from poultry to a variety of hosts.

COMPONENTS OF *SALMONELLA* BIOFILMS

All bacterial cells in a biofilm structure are embedded in a self-produced matrix of extracellular polymeric substances (EPS) [13]. The composition of EPS depends on the species and environmental conditions and can consist of a variety of polysaccharides, proteins and even nucleic acids. With regard to *Salmonella*, the essential components are cellulose [53] and curli fimbriae [5]. Cellulose (β-1-4-D glucose polymer) is an important substance in the exopolysaccharide fraction of *Salmonella* EPS and is responsible for its sticky texture and long-range cell-cell interactions [49, 53] and thus is essential for the development of *Salmonella* biofilms on epithelial cells [7, 29]. The *bcsABCZ* operon is indispensable in the expression of cellulose. Moreover, it encodes structural genes of cellulose biosynthesis [72] as well as catalytic subunit (synthase –BcsA). Using a *bcsA* mutant, it was determined that cellulose has a crucial role in biofilm formation on epithelial cell surfaces and glass [29, 45], even though Malcova et al. [33] noticed that cellulose is dispensable for *Salmonella* Enteritidis adherence to polystyrene during biofilm formation. Moreover, deficiency of cellulose in *Salmonella* Enteritidis does not affect its virulence but causes increased sensitivity to chlorine treatments [53]. While the contribution of cellulose in *Salmonella* virulence has not been researched yet, numerous studies described virulence-associated features connected with curli fimbriae expression [18, 39, 40, 51]. Their biosynthesis is performed by two operons: *csgDEFG* and *csgABC*. The main structural protein subunit of curli fimbriae is CsgA, which is positively regulated, as well as CsgB, by the global response regulator of the LuxR superfamily CsgD. CsgD protein is also required for the activation of cellulose production through the expression of AdrA, a member of the GGDEF protein family, involved in cellulose biosynthesis [72].

A hydrophobic network, consisting of cellulose and curli fimbriae covering a matrix of tightly packed *Salmonella* cells, is important in biofilm formation as well as in its survival on various biotic and abiotic surfaces [12, 53]. These facts may indicate that these EPS components play a crucial role in the persistence and resistance of *Salmonella* in food, farm and hospital environments. OMPs also play very important roles in the biofilm formations of *Salmonella* strains, because these structures are the barrier between the pathogenic cell and the host cell and they can contribute to adhesion, colonization and virulence; OMPs such as SadA are especially involved in biofilm formation, cell aggregation and increased adhesion to human intestinal epithelial cells. Another protein, such as secreted BapA protein, is also required for biofilm formation [46].

MORPHOTYPES OF *SALMONELLA* COLONY

Cellulose and/or curli fimbriae are expressed by *Enterobacteriaceae,* including *Salmonella enterica* [11, 17, 39, 47, 72]. The presence or lack of cellulose and curli fimbriae biosynthesis determines four morphotypes of *Salmonella*

colonies [49]. Expression of these EPS components may be visualized by culturing the cells of *Salmonella* serovars on Congo Red Agar at temperatures lower than 30°C [15]. *Salmonella* expressing cellulose as well as curli fimbriae display red, dry and rough (rdar) colonies. Disruption of one or both of these components leads to the development of distinct morphologies [48]. Bacteria of the pink dry and rough (pdar) morphotype express cellulose but no curli fimbriae [47, 53]. Cellulose production impairment generates a brown dry and rough (bdar) morphotype on Congo Red Agar plates, characteristic for curli fimbriae expression only. The occurrence of smooth and white (saw) colony appearance at 28°C is most likely a consequence of CsgD transcriptional regulator deactivation [47]. However, some studies [25, 56] demonstrated that anaerobic conditions affect the presence of the *Salmonella* saw morphotype at 28°C (despite the rdar mophotype occurring at the same temperature but under aerobic conditions) and inhibits biofilm production on polystyrene. In food production, a special modified atmosphere packing (MAP) is used, which decreases O_2 concentration by using CO_2. This method is widely utilized for meat preservation [71].

QUORUM-SENSING IN FORMATION OF BIOFILM STRUCTURES

The sensitivity and response to bacterial population density is a process called bacterial cell-to-cell communication or, frequently, biofilm. In some bacterial species, including *Salmonella enterica*, q*uorum sensing* regulates proper biofilm formation and pathogenicity [16, 66]. This ability could be significant in food production. The liberation of microorganisms from a biofilm, as a typical stage in its development, can initiate biofilm formation on abiotic surfaces and in consequence, contaminate produced food [26]. The *Salmonella quorum-sensing* mechanism and its vital corollary for safety of food production is researched extensively [4]. Between *Salmonella* cells, an acyl homoserine lactone (AHL*) quorum sensing* system has been recognized and described. The significant component of this system belongs to the LuxR family and is named SdiA [2]. It detects and responds to AHL signals produced by other bacterial species [1, 36, 55]. Moreover, SdiA regulates two potential *Salmonella*-loci responsible for human complement resistance, intestinal survival or colonisation: *rck* (resistance to complement killing) and srgE (*sdiA – regulated* gene E) [2, 52]. Rck promotes adherence to epithelial cells and extracellular matrix proteins. Instead, *srg* being part of the *rck* operon such as pef, appears to affect the expression and function of the *pef* operon responsible for plasmid-encoded fimbriae [8, 37].

RESISTANCE OF *SALMONELLA* IN BIOFILMS TO STRESS CONDITIONS

The essential issue in animal production and the food industry is ensuring adequate sanitation standards by regulating cleaning, appropriate disinfection and balanced use of antibiotics. It is known that bacteria in biofilms are characterized by enhanced resistance to cleaning and sanitation. Biofilms occurring in food processing environments have the potential to act as a chronic source of microbial contamination and, in consequence, foster food spoilage or disease transmission [9, 21]. Bacterial cells in biofilms differ from planktonic cells, especially in terms of their increased resistance to antimicrobials, which occurs by them using various mechanisms such as modifying their physiological state (decreased metabolism and growth rate), activating efflux pumps or multi-drug resistant operons as well as overproduction of enzymes degrading biocides and antibiotics [10]. There are many studies comparing the efficacy of commonly used disinfectants against *Salmonella* biofilm [3, 21, 23, 59]. It was noted that cellulose plays an important role in the resistance to antimicrobials by *Salmonella* biofilm. This was confirmed using cellulose mutants compared with wild-type *Salmonella* in a test of the efficacy of sodium hypochlorite [53]. Furthermore, Mangalapalli – Illathu and Korber [34] found adaptive resistance mechanism in *Salmonella* biofilms against benzalkonium chloride. Adaptation was associated with the up-regulation of key proteins involved in stress response, detoxification of cells and an overall protein biosynthesis intensification. This study showed that exposure to sub-inhibitory concentrations of disinfectants over a certain time period may cause them to acquire the ability to survive a normally lethal dose of antimicrobials, including disinfectant.

The discovery of antibiotics is one of the most significant findings in the history of medicine. Their use in clinical treatments is accompanied by the emergence of resistant bacteria, including *Salmonella*. As well as an increased resistance to disinfectants, *Salmonella* biofilms are also characterised by resistance to antibiotics. Several studies describe research on the resistance of *Salmonella* biofilm to antimicrobials, such as ampicillin, ciprofloxacin, gentamicin, tetracycline or third-generation cephalosporins such ceftriaxone and cefotaxime [32, 41]. Papavasileiou et al. [42] compared 194 *Salmonella enterica* strains isolated from hospitalized children by examining their ability to produce biofilm on silicone surfaces. Afterwards, susceptibility to nine antimicrobials was tested by comparing biofilms and planktonic forms. In every case, biofilms showed higher resistance to the tested antimicrobials, but the most significant resistance rate was for gentamicin and ampicillin. Moreover, a current problem that has been noticed concerns the increasing resistance to ciprofloxacin used to treat nontyphoid *Salmonella* infection [14]. It was reported that *S.* Typhimurium biofilms preformed on polystyrene microplates also exhibited up to a 200-fold greater resistance to ciprofloxacin compared to planktonic cells [59].

The selective pressure caused by antibiotic irresponsible use (overuse, as well as misuse), especially in veterinary medicine and animal husbandry, causes the bacteria to develop resistance [10, 14]. Furthermore, the increasing concern regarding the potential resistant bacteria transmission from the food industry via the food chain recently also includes bacteria producing biofilm [26].

ALTERNATIVE STRATEGIES TO CONTROL BIOFILM

Due to unsuccessful disinfection processes and increasing resistance to antimicrobilas among bacteria, conventional control biofilm methods, such as removing by thermal, mechanical, or chemical principles, are unfortunately becoming ineffective and new alternative startegies of biofilm eradication should be developed. Furthermore, the use of chemical detergents and disinfectants depends on their efficacy, safety and toxicity and the impact on final food product. Therefore, the use of solutions formulated with essential oils or plant extract in biofilm control process is worth considering [6].

The results of reserach conducted by Karampoula et al. [22] show significant antimicrobial action of a natural plant extract from Mediterranrean spice *Thymbra capitata* against both planktonic and biofilm *Salmonella* Typhimurium cells. According to the authors, hydrosol has numerous advantages as a disinfectant of food-contact surfaces. Next, control strategies are also described by Oh et al. [38], who tested the effect of essential oils on the anti-biological biofilm formation of *Salmonella* strains in in vitro experiments. The suppression of biofilm through essential oils has been observed. Cavacrol and thymol, which are phenolic components of oregano and thyme essential oil, had a better result on anti-biofilm formation of *Salmonella* than oregano essential oil. Confirmation of the above results can be found in the research by Trevisan et al. [62], in which it has been proved that carvacrol exhibited antibacterial and antibiofilm action against *S*. Typhimurium. Thus, it can be suggested that carvacrol could be used as an sanitization alternative for the control of bacteria in food processing environments. Furthermore, the investigation of coriander, garlic, rosemary and orange peel essential oil effects on the survival of *Salmonella* Enteritidis revealed that rosemary oil proved to be the least effective of all the tested oils [61].

The *in vitro* efficacy of natural plant extracts against biofilms calls for further research into their use in the food industry and other fields.

CONCLUSION

Studies on bacterial biofilm have been increasing due to the significance of biofilm in veterinary, clinical, environmental and food microbiology fields. Throughout this review, the profile of *Salmonella* biofilm in the food production has been elaborated. Even though many reports about this topic have been published, more studies are needed to understand properly the behaviour of *Salmonella* cells in biofilms and how wild *Salmonella* strains can survive within the whole food chain. Unquestionably, any assays studying commonly used nutrients, such as fruit, vegetables or meat juices in biofilm formation, provide practical knowledge and should be constantly conducted and improveed. Furthermore, all phenotypic biofilm assays should be enriched by transcriptomic and proteomic studies. The success of alternative methods in inhibiting cell attachment and biofilm development indicates a promising implement for reducing microbial colonization of food processing surfaces. Furthermore, understanding all issues discussed above allows for the control and even prevention of biofilm formation in the food industry.

REFERENCES

[1] Ahmer B.M.: Cell-to-cell signalling in *Escherichia coli* and *Salmonella enterica*. Mol. Microbiol., 2004; 52: 933–945

[2] Ahmer B.M., van Reeuwijk J., Timmers C.D., Valentine P.J., Heffron F.: *Salmonella typhimurium* encodes a SdiA homolog, a putative quorum sensor of the LuxR family, that regulates genes on the virulence plasmid. J. Bacteriol., 1998; 180: 1185–1193

[3] Arnold J.W., Yates I.E.: Interventions for control of Salmonella: Clearance of microbial growth from rubber picker fingers. Poult. Sci., 2009; 88: 1292–1298

[4] Bai A.J., Rai V.R.: Bacterial quorum sensing and food industry. Compr. Rev. Food Sci. Food Saf., 2011; 10: 183–193

[5] Barnhart M.M., Chapman M.R.: Curli biogenesis and function. Annu. Rev. Microbiol., 2006; 60: 131–147

[6] Bazargani M.M., Rohloff J.: Antibiofilm activity of essential oils and plant extracts against *Staphylococcus aureus* and *Escherichia coli* biofilms. Food Control, 2016; 61: 156–164

[7] Boddicker J.D., Ledeboer N.A., Jagnow J., Jones B.D.: Clegg, S.: Differential binding to and biofilm formation on, Hep-2 cells by *Salmonella enetrica* Serovar Typhimurium is dependent upon allelic variation in the *fimH* gene of the *fim* gene cluster. Mol. Microbiol., 2002; 45: 1255–1265

[8] Bouwman C.W., Kohli M., Killoran A., Touchie G.A., Kadner R.J., Martin N.L.: Characterization of SrgA, a *Salmonella enetrica* Serovar Typhimurium virulence plasmid-encoded paralogue of the disulphide oxidoreductase DsbA, essential for biogenesis of plasmid-encoded fimbriae. J. Bacteriol., 2003; 185, 991–1000

[9] Bower C.K., Daeschel M.A.: Resistance responses of microorganisms in food environments. Int. J. Food Microbiol., 1999; 50: 33–44

[10] Capita R., Alonso-Calleja C.: Antibiotic-resistant bacteria: A challenge for the food industry. Crit. Rev. Food Sci. Nutr., 2013; 53: 11–48

[11] Collinson S.K., Clouthier S.C., Doran J.L., Banser P.A., Kay W.W.: *Salmonella enteritidis agfBAC* operon encoding thin, aggregative fimbriae. J. Bacteriol., 1996; 178: 662–667

[12] Cookson A.L., Cooley W.A., Woodward M.J.: The role of type 1 and curli fimbriae of Shiga toxin-producing *Escherichia coli* in adherence to abiotic surfaces. Int. J. Med. Microbiol., 2002; 292: 195–205

[13] Costerton J.W., Lewandowski Z., Caldwell D.E., Korber D.R., Lappin-Scot H.M.: Microbial biofilms. Annu. Rev. Microbiol., 1995; 49: 711–745

[14] European Food Safety Authority, European Centre for Disease Prevention and Control: The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2016. EFSA J., 2017; 15: 5077

[15] Gerstel U., Park C., Römling U.: Complex regulation of csgD promoter activity by global regulatory proteins. Mol. Microbiol., 2003; 49: 639–654

[16] Halatasi K., Oikonomou I., Lambiri M., Mandilara G., Vatopoulus, A., Kyriacou A.: PCR detection *Salmonella* spp. using primers targeting the quorum sensing gene *sdiA*. FEMS Microbiol. Lett., 2006; 259: 201–207

[17] Hammar M., Bian Z., Normark S.: Nucleator-dependent intercellular assembly of adhesive curli organelles in Escherichia coli. Proc. Natl. Acad. Sci. USA, 1996; 93: 6562–6566

[18] Herwald H., Mörgelin M., Olsén A., Rhen M., Dahlbäck B., Müller-Esterl W., Björck L.: Activation of the contact-phase system on bacterial surfaces – a clue to serious complications in infection diseases. Nat. Med., 1998; 4: 298–302

[19] Hood S.K., Zottola E.A.: Adherence to stainless steel by foodborne microorganisms during growth in model food systems. Int. J. Food Microbiol., 1997; 37: 145–153

[20] Janssens J.C., Steenackers H., Robbijns S., Gellens E., Levin J., Zhao H., Hermans K., De Coster D., Verhoeven T.L., Marchal K., Vanderleyden J., De Vos D.E., De Keersmaecker S.C.: Brominated furanones inhibit biofilm formation of *Salmonella enterica* serovar Typhimurium. Appl. Enviorn. Microbiol., 2008; 74: 6639–6648

[21] Joseph B., Otta S.K., Karunasagar I., Karunasagar I.: Biofilm formation by *Salmonella* spp. on food contact surfaces and their sensitivity to sanitizers. Int. J. Food Microbiol., 2001; 64: 367–372

[22] Karampoula F., Giaouris E., Deschamps J., Doulgeraki A.I., Nychas G.J., Dubois-Brissonnet F.: Hydrosol of *Thymbra capitata* is a highly efficient biocide against *Salmonella enetrica* serowar Typhimurium biofilms. Appl. Environ Microbiol., 2016; 82: 5309–5319

[23] Korber D.R.; Choi A., Wolfaardt G.M., Ingham S.C., Caldwell D.E.: Substratum topography influences susceptibility of Salmonella enteritidis biofilms to trisodium phosphate. App. Environ. Microbiol., 1997; 63: 3352–3358

[24] Lamas A., Fernandez-No I.C., Miranda J.M., Vázquez B., Cepeda A., Franco C.M.: Biofilm formation and morphotypes of *Salmonella enterica* subsp. *arizonae* differs from those of other *Salmonella enterica* subspecies in isolates from poultry houses. J. Food. Prot., 2016; 79: 1127–1134

[25] Lamas A., Miranda J.M., Vázquez B., Cepeda A., Franco C.M.: Biofilm formation, phenotypic production of cellulose and gene expression in *Salmonella enterica* decrease under anaerobic conditions. Int. J. Food. Microbiol., 2016; 238: 63–67

[26] Lamas A., Regal P., Vázquez B., Miranda J.M., Cepeda A., Franco C.M.: *Salmonella* and *Campylobacter* biofilm formation: a comparative assessment from farm to fork. J. Sci. Food Agric., 2018; 98: 4014–4023

[27] Latasa C., Roux A., Toledo-Arana A., Ghigo J.M., Gamazo C., Penadés J.R., Lasa I.: BapA, a large secreted protein required for biofilm formation and host colonization of *Salmonella enterica* serovar Enteritidids. Mol. Microbiol., 2005; 58: 1322–1339

[28] Ledeboer N.A., Frye J.G., McClelland M., Jones B.D.: *Salmonella enetrica* serovar Typhimurium requires the Lpf, Pef and Tafi fimbriae for biofilm formation on Hep-2 tissue culture cells and chicken intestinal epithelium. Infect. Immun., 2006; 74: 3156–3169

[29] Ledeboer N.A., Jones B.D.: Exopolysaccharide sugars contribute to biofilm formation by *Salmonella enterica* serovar Typhimurium on Hep-2 cells and chickens intestinal epithelium. J. Bacteriol., 2005; 187: 3214–3226

[30] Lianou A., Koutsoumanis K.P.: Strain variability of the biofilm-forming ability of *Salmonella enterica* under various environmental conditions. Int. J. Food Microbiol., 2012; 160: 171–178

[31] MacKenzie K.D., Palmer M.B., Köster W.L., White A.P.: Examining the link between biofilm formation and the ability of pathogenic *Salmonella* strains to colonize multiple host species. Front. Vet. Sci., 2017; 4: 138

[32] Majtán J., Majtánová L., Xu M., Majtán V.: *In vitro* effects subinhibitory concentrations of antibiotics on biofilm formation by clinical strains of *Salmonella enetrica* serovar Typhimurium isolates in Slovakia. J. App. Mirobiol., 2008; 104: 1294–1301

[33] Malcova M., Karasova D., Rychlik I.: *aroA* and *aroD* mutations influence biofilm formation in *Salmonella* Enteritidis. FEMS Microbiol. Lett., 2009; 291: 44–49

[34] Mangalappalli-Illathu A.K., Korber D.R.: Adaptive resistance and differential protein expression of *Salmonella enterica* serovar Enteritidis biofilms exposed to benzalkonium chloride. Antimicrob. Agents Chemother., 2006; 50: 3588–3596

[35] Markland S., Ingram D., Kniel K.E., Sharma M.: Water for agriculture: The convergence of sustainability and safety. Microbiol. Spectr., 2017; 5: PFS-0014–2016

[36] Michael B., Smith J.N., Swith S., Heffron F., Ahmer B.M.: SdiA of *Salmonella enterica* is a LuxR homolog that detects mixed microbial communities. J. Bacteriol., 2001; 183: 5733–5742

[37] Nicholson B., Low D.: DNA methylation-dependent regulation of pef expression in *Salmonella typhimurium*. Mol. Microbiol., 2000; 35: 728–742

[38] Oh S.Y., Yun W., Lee J.H., Lee C.H., Kwak W.K. Cho J.H.: Effects of essential oil (blended and single essential oils) on anti-biofilm formation of *Salmonella* and *Escherichia coli*. J. Anim. Sci. Technol., 2017; 59: 4

[39] Olsén A., Herwald H., Wikström M., Persson K., Mattson E., Björck L.: Identification of two protein-binding and functional regions of curli, a surface organelle and virulence determinant of *Escherichia coli*. J. Biol. Chem., 2002; 277: 34568–34572

[40] Olsén A., Jonsson A., Normark S.: Fibronectin binding mediated by a novel class of surface organelles on *Escherichia coli*. Nature, 1989; 338: 652–655

[41] Olson M.E., Ceri H., Morck D.W., Buret A.G., Read R.R.: Biofilm bacteria: formation and comparative susceptibility to antibiotics. Can. J. Vet. Res., 2002; 66: 86–92

[42] Papavasileiou K., Papavasileiou E., Tseleni-Kotsovii A., Bersimis S., Nicolaou C. Ioannidis A., Chatzipanagiotou S.: Comparative antimicrobial susceptibility of biofilm versus planktonic forms of *Salmonella enterica* strains isolated from children with gastroenteritis. Eur. J. Clin. Microbiol. Infect. Dis., 2010; 29: 1401–1405

[43] Paz-Méndez A.M., Lamas A., Vázquez B., Miranda J.M., Cepeda A., Franco C.M.: Effect of food residues in biofilm formation on stainless steel and polystyrene surfaces by *Salmonella enterica* strains isolated from poultry houses. Foods, 2017; 6: E106

[44] Proctor M.E., Hamacher M., Tortorello M.L., Archer J.R., Davis J.P.: Multistate outbreak of *Salmonella* serovar Muenchen infections associated with alfalfa sprouts grown from seeds pretreated with calcium hypochlorite. J. Clin. Microbiol., 2001; 39: 3461–3465

[45] Prouty A.M., Gunn J.S.: Comparative analysis of *Salmonella enterica* serovar Typhimurium biofilm formation on gallstones and on glass. Infect. Immun., 2003; 71: 7154–7158

[46] Raghunathan D., Wells T.J., Morris F.C., Shaw R. Bobat K., Peters S.E., Paterson G.K., Jensen K.T., Leyton D.L., Blair J.M., Browning D.F., Pravin J., Flores-Langarica A., Hitchcock J.R., Moraes C.T., et al.: SadA, a trimeric autotransporter from *Salmonella enterica* serovar Typhimurium, can promote biofilm formation and provides limited protection against infection. Infect. Immun., 2011; 79: 4342–4352

[47] Römling U.: Molecular biology of cellulose production in bacteria. Res. Microbiol., 2002; 153: 205–212

[48] Römling U., Bokranz W., Rabsch W., Zogaj X., Nimtz M., Tschäpe H.: Occurence and regulation of the multicellular morphotype in *Salmonella* serovars important in human disease. Int. J. Med. Microbiol., 2003; 293: 273–285

[49] Römling U., Rhode M., Olsén, A., Normark S., Reinköster J.: *AgfD*, the checkpoint of multicellular and aggregative behaviour in *Salmonella typhimurium* regulates at least two independent pathways. Mol. Microbiol., 2000; 36: 10–23

[50] Sivapalasingam S., Barrett E., Kimura A., Van Duyne S., De Witt W., Ying M., Frisch A., Phan Q., Gould E., Shillam P., Reddy V., Cooper T., Hoekstra M., Higgins C., Sanders J.P., et al.: A multistate outbreak of *Salmonella enterica* Serotype Newport infection linked to mango consumption: Impact of water-dip disinfestation technology. Clin. Infect. Dis., 2003; 37: 1585–1590

[51] Sjöbring U., Pohl G., Olsén A.: Plasminogen, absorbed by Escherichia coli expressing curli or by Salmonella enteritidis expressing thin aggregative fimbriae, can be activated by simultaneously captured tissue-type plasminogen activator (t-PA). Mol. Microbiol., 1994; 14: 443–452

[52] Smith J.N., Ahmer B.M.: Detection of other microbial species by *Salmonella*: Expression of the SdiA regulon. J. Bacteriol., 2003; 185: 1357–1366

[53] Solano C., Garcia B., Valle J., Berasain C., Ghigo J.M., Gamazo C., Lasa I.: Genetic analysis of *Salmonella enteritidis* biofilm formation: critical role of cellulose. Mol. Microbiol., 2002; 43: 793–808

[54] Solomon E.B., Niemira B.A., Sapers G.M., Annous B.A.: Biofilm formation, cellulose production, and curli biosynthesis by *Salmonella* originating from produce, animal, and clinical sources. J. Food Prot., 2005; 68: 906–912

[55] Sperandio V., Torres A.G, Kaper J.B.: Quorum sensing *Escherichia coli* regulators B and C (QseBC): A novel two-component regulatory system involved in the regulation of flagella and motility by quorum sensing in *E. coli*. Mol. Microbiol., 2002; 43: 809–821

[56] Steenackers H., Hermans K., Vanderleyden J., De Keersmaecker S.C.J.: *Salmonella* biofilms: An overview on occurrence, structure, regulation and eradication. Food Res. Inter., 2012; 45: 502–531

[57] Stein R.A.: Super-spreaders in infectious diseases. Int. J. Infect. Dis., 2011; 15: e510–e513

[58] Stepanović S., Cirković L., Ranin L., Svabić-Vlahović M.: Biofilm formation of *Salmonella* spp. and *Listeria monocytogenes* on plastic surface. Lett. App. Microbiol., 2004; 38: 428–432

[59] Tabak M., Scher K., Chikindas M.L., Yaron S.: The synergistic activity of triclosan and ciprofloxacin on biofilms of *Salmonella* Typhimurium. FEMS Microbiol. Lett., 2009; 301: 69–76

[60] Tabak M., Scher K., Hartog E., Romling U., Matthews K.R., Chikindas M.L., Yaron S.: Effects of triclosan on *Salmonella typhimurium* at different growth stages and in biofilms. FEMS Microbiol. Lett., 2007; 267: 200–206

[61] Tosun S.Y., Alakavuk D.U., Ulusoy S., Erkan N.: Effects of essential oils on the survival of *Salmonella* Enteritidis and *Listeria monocytogenes* on fresh Atlantic salmons (Salmo salar) during storage at 2±1 °C. J. Food Saf., 2017; 38: e12408

[62] Trevisan D.A., da Silva A.F., Negri M., de Abreu Filho B.A., Machinski Junior M., Patussi E.V., Campanerut-Sá P.A., Mikcha J.M.: Antibacterial and antibiofilm activity of carvacrol against Salmonella enterica serotype Typhimurium. Braz. J. Pharm. Sci., 2018; 54: e17229

[63] Vestrheim D.F., Lange H., Nygård K., Borgen K., Wester A.L., Kvarme M.L., Vold L.: Are ready-to-eat salads ready to eat? An outbreak of *Salmonella* Coeln linked to imported, mixed, pre-washed and bagged salad, Norway, November 2013. Epidemiol. Infect., 2016; 144: 1756–1760

[64] Wadamori Y., Gooneratne R., Hussain M.A.: Outbreaks and factors influencing microbiological contamination of fresh produce. J. Sci. Food Agric., 2017; 97: 1396–1403

[65] White A.P. Surette M.G.: Comparative genetics of the rdar morphotype in *Salmonella*. J. Bacteriol., 2006; 188: 8395–8406

[66] Williams P.: Quorum sensing. Int. J. Med. Microbiol., 2006; 296: 57–59

[67] Wong A.C.: Biofilms in food processing environments. J. Dairy Sci., 1998; 81: 2765–2770

[68] Woolhouse M.E., Dye C., Etard J.F., Smith T., Charlwood J.D., Garnett G.P., Hagan P., Hii J.L., Ndhlovu P.D., Quinnell R.J., Watts C.H., Chandiwana S.K., Anderson R.M.: Heterogeneities in the transmission of infectious agents: implications for the design of control programs. Proc. Natl. Acad. Sci. USA, 1997; 94: 338–342

[69] Xavier J.B., Foster K.R.: Cooperation and conflict in microbial biofilms. Proc. Nat. Acad. Sci. USA, 2007; 104: 876–881

[70] Yaron S., Römling U.: Biofilm formation by enteric pathogens and its role in plant colonization and persistence. Microb Biotechnol., 2014; 7: 496–516

[71] Zhou G.H., Xu X.L., Liu Y.: Preservation technologies for fresh meat – a review. Meat Sci., 2010; 86: 119–128

[72] Zogaj X., Nimtz M., Rohde M., Bokranz W., Römling U.: The multicellular morphotypes of *Salmonella typhimurium* and *Escherichia coli* produce cellulose as the second component of the extracellular matrix. Mol. Microbiol., 2001; 39: 1452–1463

The authors have no potential conflicts of interest to declare.