We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,300

Open access books available

172,000

International authors and editors

190M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

Biofilms Formed by Pathogens in Food and Food Processing Environments

*Leontina Grigore-Gurgu, Florentina Ionela Bucur,
Daniela Borda, Elena-Alexandra Alexa, Corina Neagu
and Anca Ioana Nicolau*

Abstract

This chapter presents the ability of some pathogenic (*Listeria monocytogenes*, *Escherichia coli*, *Salmonella enterica*, *Campylobacter jejuni*, *Pseudomonas aeruginosa*) and toxigenic bacteria (*Bacillus cereus*, *Staphylococcus aureus*) to form biofilms and contribute to the persistence of these microorganisms in the food industry. Particularities regarding attachment and composition of biofilms formed in food and food processing environments are presented and genes involved in biofilm production are mentioned. To give a perspective on how to fight against biofilms with new means, nonconventional methods based on bacteriocins, bacteriophages, disruptive enzymes, essential oils, nanoemulsions and nanoparticles, and use of alternative technologies (cold plasma, ultrasounds, light-assisted technologies, pulsed electric field, and high pressure processing) are shortly described.

Keywords: bacteriocin, essential oils, bacteriophages, nanoemulsion, alternative technologies

1. Introduction

Food matrices having water activities above 0.9 and wet food processing environments are wonderlands for microorganism multiplication and biofilm development. Biofilms are considered of great concern in regard to functioning of mechanical parts that may be blocked, to energy consumption, which becomes higher when heat transfer decreases, and to corrosion as corrosion rate of surfaces increases underneath biofilms (corrosion grows 10–1000 times faster causing loss of material and increasing porosity) but their presence in food and food processing environments is also a serious public health risk due to problems associated with foodborne illnesses and food spoilage [1].

The biofilms that are threatening the safety of food products are produced by some pathogenic bacteria such as *Listeria monocytogenes*, *Escherichia coli*, *Salmonella enterica*, *Campylobacter jejuni*, and *Pseudomonas aeruginosa* and toxigenic bacteria such as *Staphylococcus aureus* and *Bacillus cereus* [2]. Biofilms are responsible for persistence of such bacteria in food processing environments and (re)contamination

of processed foods [3]. When contamination of food products happens, recalls are necessary. These actions present large economic burden to industry and are also associated with brand damage.

2. Biofilm formation

Biofilms are formed on all types of surfaces existing in food plants ranging from plastic, glass, metal, cement, to wood and food products [4]. Usually, biofilms form a monolayer or more often multilayers, in which bacteria may undergo a significant change in physiology with an increased tolerance to environmental stresses [5].

L. monocytogenes, the pathogen that proliferates at low temperatures, is able either to form pure culture biofilms or to grow in multispecies biofilms [6]. Prevalent strains in food processing environments have good adhesion ability due to the presence of flagella, pili, and membrane proteins [7]. Composition of biofilms produced by *L. monocytogenes* is different in comparison with that produced by other bacteria. For example, exopolysaccharides like alginate in *Pseudomonas* or poly-N-acetylglucosamine in *Staphylococcus* have not been put into evidence [8].

Salmonella spp. express proteinaceous extracellular fibers called curli that are involved in surface and cell-cell contacts and promotion of community behavior and host colonization [9]. Besides curli, different fimbrial adhesins have been identified to have implications in biofilm formation, dependent of serotype. The presence of cellulose in the biofilm matrix contributes to cells' resistance to mechanical forces and improved adhesion to abiotic surfaces [10]. Significant differences between serovars were put into evidence regarding biofilm formation the most persistent in food processing environments being the ones that are capable to form biofilms [11].

Flagella, pili, and membrane proteins are also used by *E. coli* to initiate attachment on inanimate surfaces. Flagella are lost after attachment and bacteria start producing an extracellular polymeric substance (EPS) that provides a better resistance of bacteria to disinfectants as hypochlorite [12]. Similarities in biofilm structure and composition as well as regulatory mechanisms with *Salmonella* spp. have been demonstrated for *E. coli*, mostly in terms of expression of small RNAs leading to a change in bacterial physiology regarding the cell motility and production of curli or EPS [13].

In general terms, different *E. coli* serotypes have been reported to enhance flexibility and adaptability in forming biofilms when exposed to different stresses. For example, *E. coli* seropathotype A isolates associated with human infection, O157:H7 and O157:NM, showed greater ability to form biofilms than those belonging to seropathotype B or C associated with outbreaks and hemolytic-uremic syndrome (HUS) or sporadic HUS cases but no epidemics, respectively [14]. In addition, synergistic interactions are taking place in a fresh-cut produce processing plant in which *E. coli* is interacting with *Burkholderia caryophylli* and *Ralstonia insidiosa* with the formation of mixed biofilms [15].

C. jejuni, which is known as an anaerobic bacterium, is able to develop biofilms both in microaerophilic conditions (5% O₂ and 10% CO₂) and in aerobic conditions (20% O₂) [1]. The cells embedded in the biofilm matrix are better protected from oxygen and survive for days in food processing environments [1].

Pseudomonas spp. produce high amounts of EPS and have been shown to attach and form biofilms on stainless steel surfaces. They coexist within biofilms with *Listeria*, *Salmonella*, and other pathogens forming multispecies biofilms, more stable and resistant [6].

B. cereus is a cause of biofilm formation on many food contact surfaces such as conveyor belts, stainless steel pipes, and storage tanks [16], but it is also able to

form immersed or floating biofilms, and to secrete within the biofilm a vast array of metabolites, surfactants, bacteriocins, enzymes as lipases and proteases affecting the sensorial qualities of foods, and toxins. For floating biofilms, the production of kurstakin, a lipopeptide biosurfactant, that is regulated via quorum sensing (QS) signaling is important [17].

Within the biofilm, *B. cereus* exists either in vegetative or in sporal form, the spores being highly resistant and adhesive, properties that increase the resistance of the bacterium to antimicrobials and cleaning procedures.

Four mechanisms based on the flagellar motility of *B. cereus* are described as being involved in biofilm formation. The first mechanism is used in static conditions when the bacterium must reach on its own suitable places for biofilm formation [18], at the air-liquid interface. The second one is represented by the creation of channels in the biofilm matrix to facilitate nutrients' access on one hand and penetration of toxic substances on the other hand [19]. The third mechanism refers to motile planktonic bacteria that penetrate the biofilm and increase its biomass [18, 19], while the fourth represents the extension of the biofilm based on the ability of motile bacteria located at the edge of the biofilm to colonize the surroundings [18].

It has been showed that, in its planktonic form, *S. aureus* does not appear resistant to disinfectants, compared to other bacteria, but it may be among the most resistant ones when attached to a surface [20]. It seems that different stress-adaptive responses may enhance biofilm formation, with certain differences in terms of their composition and architecture, especially for the wild-type biofilms colonizing the food and related processing environments. Examples include protein-based sources responsible for the structure of biofilms formed by *S. aureus* of food origin [21] similar to those put into evidence for the coagulase-negative ones. However, other studies demonstrated that simple carbohydrates, such as milk lactose, can modulate the biofilm formation especially by inducing the production of polysaccharide intercellular adhesins [22].

3. Genes involved in the biofilm formation

Over time, beside the conditions that favor the biofilm formation in food processing plants, the genetic background of biofilm forming microorganisms was also intensively studied. At each step of biofilm development and dispersal, there is a specific genetic signal control.

The *L. monocytogenes* pattern of the microarray gene expression was analyzed at different time intervals (4, 12, and 24 h) in order to depict genes' expression at different stages of biofilm formation. The results showed that more than 150 genes were upregulated after 4 h of biofilm formation and a total of 836 genes highlighted a slow increase in expression with time [23]. Although for many bacterial species the genome sequencing allowed the identification of genes that were involved in biofilm synthesis, for *L. monocytogenes*, these genes could not be identified using just the bioinformatics analysis.

In the biofilm formation, the attachment step is a prerequisite in which flagella and type I pili-mediated motilities are critical for the initial interaction between the cells and surface.

In order to find out the roles of the genes and regulatory pathway controlling the biofilm formation, researchers applied one or two genome-wide approaches, like transposon insertion mutagenesis or/and transcriptome analyses. With a transposon mutagenesis library, it was possible to identify 70 *L. monocytogenes* mutants, with Himar1 mariner transposon insertion, which produced less biofilms [24]. From a total of 38 genetic loci identified, 4 of them (**Table 1**) were found to be involved in bacterial motility (*fliD*, *fliQ*, *flaA*, and *motA*), a required property for initial surface

Gene/KEGG/protein encoded	Gene function	Role	Bacterium	Ref.
Initial attachment				
<i>fliQ</i> /LMON_0682/Flagellar biosynthesis protein	Motility	Cell adhesion and bacterial attachment	<i>L. monocytogenes</i>	[23–25]
<i>flaA</i> /lmo0690/Flagellin	Flagella bio-synthesis			
<i>fliD</i> /Flagellar hook-associated protein 2	Enable the polymerization of the flagellin monomers; flagellar capping protein			
<i>motA</i> /BN418_0793/Flagellar motor protein	Flagellar motor rotation			[148]
<i>prfA</i> /IJ09_09365/Listeriolysin positive regulatory factor A	DNA-binding transcription factor activity	Positive regulation of single species biofilm formation	<i>L. monocytogenes</i>	
<i>fimA</i> /JW4277/Type-1 fimbrial protein, A chain	Enable bacteria to colonize the host epithelium	Cell adhesion	<i>E. coli</i>	[30, 31]
<i>fliA</i> /ECUMN_0250/Flagellar biosynthesis protein	Motility bacterial-type flagellum assembly			
<i>yadL</i> /ECs0141/ <i>yadM</i> / <i>yadK</i> / <i>yadC</i> /Fimbrial protein	Fimbrial bio-synthesis			
<i>tabA</i> / <i>yjgK</i> /b4252/toxin-antitoxin biofilm protein	Represses fimbria genes	Single-species biofilm		
<i>icaA</i> /Poly-beta-1,6-N-acetyl-D-glucosamine synthase from <i>ica</i> ADBCR operon	Acetylglucosaminyl transferase activity, cell adhesion	Involved in the polymerization of a biofilm adhesin polysaccharide	<i>S. aureus</i>	[149]
<i>tpiA</i> /SAR0830/Triosephosphate isomerase	Involved in gluconeogenesis pathway	Role in adherence		[150]
<i>sraP</i> /SAOUHSC_02990/ Serine-rich adhesin for platelets	Mediates binding to human platelets	Plays a positive role in biofilm formation		[151]
<i>Spo0A</i> /BSU24220/Stage 0 sporulation protein A	Regulatory role in sporulation	Single-species surface biofilm formation	<i>B. cereus</i> , <i>B. subtilis</i>	[152] [153]
<i>degS</i> /BSU35500/Signal transduction histidine-protein kinase/phosphatase	Transition to growth phase; flagellum formation	Biofilm formation		[154, 155]
<i>fliL</i> /STM1975/Flagellar protein	Controls the rotational direction of flagella	Motility, cell adhesion	<i>S. enterica</i>	[156]
<i>ycfR</i> /Outer membrane protein	Promotes the attachment to the surface			[157]

Gene/KEGG/protein encoded	Gene function	Role	Bacterium	Ref.
Microcolonies development				
<i>dltA</i> /LMOF2365_099/D-alanine-D-alanyl carrier protein ligase	Catalyzes the first step in the D-alanylation of lipoteichoic acid (LTA)	Cell wall biogenesis	<i>L. monocytogenes</i> , <i>S. aureus</i>	[24]
<i>dltC</i> /LMOF2365_099/D-alanyl carrier protein	Carrier protein involved in the D-alanylation of LTA			
<i>dltB</i> /lmo0973/DltB	Involved in the transport of activated D-alanine through the membrane		<i>S. aureus</i> , <i>B. subtilis</i>	
<i>sdrC</i> /NWMN_0523/Serine-aspartate repeat-containing protein C <i>sdrH</i> /SAUSA300_1985 Serine-aspartate repeat family protein	Cell adhesion	Mediates interactions with components of the extracellular matrix to promote bacterial adhesion	<i>S. aureus</i>	[158]
<i>bhsA</i> /STY1254/Multiple stress resistance protein	Stress response, response to copper ion	Regulation of biofilm formation. May repress cell–cell interaction and cell surface interaction	<i>E. coli</i>	[159]
<i>bsmA</i> /yjfO/Lipoprotein	Stress response to hydrogen peroxide and to DNA damage	Single-species biofilm formation; enhanced flagellar motility	<i>E. coli</i> , <i>S. enterica</i>	[160]
<i>csgD</i> /b1040/CsgBAC operon transcriptional regulatory protein	DNA-binding transcription activator activity	The master regulator for adhesive curli fimbriae expression		[161]
<i>mlrA</i> /b2127/HTH-type transcriptional regulator	DNA-binding transcription factor activity	Activates transcription of <i>csgD</i>		[162]
<i>simR</i> /BSU24610/HTH-type transcriptional regulator	Negatively regulates transcription of the <i>eps</i> operon	DNA-binding protein master regulator of biofilm formation	<i>B. subtilis</i> , <i>B. cereus</i>	[163, 164]
<i>epsG</i> (<i>yveQ</i>)/BSU34310/Transmembrane protein	Production of exopolysaccharide	Biofilm maintenance		[165]
<i>epsH</i> (<i>yveR</i>)/BSU34300/Putative glycosyl-transferase				[166]
<i>ymdB</i> /BSU16970/2',3'-cyclic-nucleotide 2'-phospho-diesterase	Regulatory role. Induces genes involved in biofilm formation			
<i>pgcA</i> /Phosphoglucumutase	Catalyzes the interconversion between glucose-6-phosphate and alpha-glucose-1-phosphate	Exopolysaccharide synthesis		[167]

Gene/KEGG/protein encoded	Gene function	Role	Bacterium	Ref.
<i>gcpA</i> /SL1344_191/Biofilm formation in nutrient-deficient medium	Biofilm production under low-nutrient concentrations		<i>S. enterica</i>	[156]
Biofilm maturation				
<i>tasA</i> /BSU24620/major biofilm matrix component	Identical protein binding	Major component of the biofilm extracellular matrix	<i>B. cereus</i>	[168]
<i>tapA</i> /BSU24640/TasA anchoring/assembly protein	Important for proper anchoring and polymerization of TasA fibers at the cell surface	Essential for biofilm formation	<i>B. subtilis</i> No paralog in <i>B. cereus</i> genome	[169]
<i>sipW</i> /BSU24630/Signal peptidase IW	Cleavage of the signal sequence of TasA and TapA		<i>B. cereus</i>	
<i>bslA</i> (<i>yuaB</i>)/BSU31080/Biofilm-surface layer protein A	Confers a specific microstructure to the biofilm surface	Confers hydrophobicity to the biofilm	<i>B. subtilis</i> , No paralog in <i>B. cereus</i> genome	[170–171]
<i>wcaF</i> /b2054/Putative colanic acid biosynthesis acetyl-transferase	Synthesis of colanic acid	Involved in the pathway slime polysaccharide biosynthesis	<i>E. coli</i>	[172]
<i>wcaL</i> /STM2100/Putative colanic acid biosynthesis glycosyl-transferase			<i>S. enterica</i>	[173]
<i>bssR</i> (<i>yliH</i>)/JW0820/Biofilm regulator	Regulation of biofilm formation	In the glucose presences, cells showed increased biofilm formation	<i>E. coli</i>	[33]
<i>mqsR</i> /b3022/mRNA interferase toxin	Motility-quorum sensing cell proliferation	Biofilm architecture		[172]
<i>tqsA</i> /b1601/AI-2 transport protein	Efflux transmembrane transporter activity	Represses biofilm formation and motility		[31]
<i>bdcA</i> /b4249/Cyclic-di-GMP-binding biofilm dispersal mediator protein	Controls cell motility, size, aggregation, and production of extracellular DNA and extracellular polysaccharides	Biofilm dispersal	<i>E. coli</i> , <i>S. enterica</i>	[174]
<i>ihfAB</i> /Integration host factor	Specific DNA-binding protein	Matrix density Cellulose production	<i>S. enterica</i> , <i>S. aureus</i>	[175–177]
<i>bapA</i> /biofilm-associated protein	Large surface proteins family	Bacterial adhesion Biofilm maturation		

Gene/KEGG/protein encoded	Gene function	Role	Bacterium	Ref.
<i>clfA</i> / / Clumping factor A; <i>clfB</i> / NWMN_2529/ Clumping factor B	Cell surface-associated protein implicated in bacterial attachment	Aggregation of unicellular organisms; cell adhesion	<i>S. aureus</i>	[178]
<i>icaC</i> /SAOUHSC_03005/poly-beta-1,6-N-acetyl-D-glucosamine export protein (PNAG)	Export of PNAG across the cell membrane		<i>E. coli</i> , <i>S. aureus</i>	[149]
<i>pflA</i> /SAOUHSC_00188/Pyruvate formate lyase-activating enzyme <i>pflB</i> /SACOL020/Formate acetyltransferase	Enzymes that catalyze the first step in the acetogenesis from pyruvate	Organic free radical synthesis		[29]
<i>sarA</i> /Transcriptional regulator	Global regulator of a few genes with important roles in biofilm development	Biofilm formation process in a cell density-dependent manner	<i>S. aureus</i>	[179]
<i>agrD</i> /LMM7_0043/Putative autoinducing peptide	Involved in proteolytic processing	Quorum Sensing	<i>L. monocytogenes</i>	[180]
lmo0048/Putative AgrB-like protein	Involved in proteolytic processing		<i>L. monocytogenes</i> <i>B. cereus</i>	
<i>agrC</i> /Accessory gene regulator	Histidine kinase activity		<i>S. aureus</i>	[181]
<i>agrA</i> /CQ02_00305/BN389_00610/ Accessory gene regulator	A response regulator			[182] [183]
<i>agrB</i> /MF_00784/Accessory gene regulator	Proteolytic processing of AgrD		<i>S. aureus</i>	[184]
<i>luxS</i> /lmo1288/S-ribosyl-homo-cysteine lyase	Catalysis of precursor molecules of AI-2		<i>L. monocytogenes</i> <i>E. coli</i> , <i>B. cereus</i> , <i>S. enterica</i>	[48] [49]
<i>luxQ</i> /Autoinducer 2 sensor kinase/phosphatase	Phospho-relay sensor kinase activity		<i>E. coli</i> , <i>B. cereus</i> , <i>S. enterica</i>	

Table 1.
List of genes with significant role in biofilm formation within pathogenic microorganisms (UniprotKB database).

attachment. Another gene with increased expression at 4 h and decreased expression after 12 h from biofilm initiation was *prfA*, the listeriolysin positive regulatory factor A. It seems that this regulatory factor is necessary just in the initial stages of biofilm formation and aggregation but not in the colonization stage [23, 25, 26].

Extracellular and surface proteins such as internalin A and BapL, respectively, have been found to be involved in the initial bacterial adhesion in *L. monocytogenes* EGD-e [27]. Moreover, its mobility is ensured by flagella and is temperature-dependent affecting the biofilm formation. As such, above 30°C, the transcription of *flaA* is stopped.

S. aureus genes responsible for cell adhesion to the surface are included in the *icaADBC* operon with functions in biosynthesis of the glucosamine polymer and polysaccharide intercellular adhesins [28]. Therefore, other genes encoding a number of transporter proteins (*proP*, *opuD*, *aapA*, and *dltA*) were upregulated after 8 hours from the biofilm initiation [29]. For *E. coli*, the genes involved in the cell adhesion, like *fimA*, *yadK*, *yadN*, *yadM*, and *yadC*-encoding fimbriae-like proteins-are coexpressed with the integral cell membrane genes, with outer membrane proteins (*htrE*), with transcriptional regulators (*mngR* and *nhaR*), or other genes, but this network appears to be strain specific [30, 31].

In the case of *S. enterica*, differential expression analysis revealed that *yefR* is highly conserved as in many Gram-negative bacteria, being upregulated under chlorine stress and responsible for the virulence and attachment of bacterium to the glass or polystyrene [32, 33].

Moreover, *Salmonella* spp.-related biofilms are driven by a transcriptional regulatory CsgD protein that activates the expression of curli and cellulose. The transcription of *csgBAC* operon, which encodes the structural subunits for curli, indirectly activates the transcription of the second mechanism, *adrA*, associated with cellulose production [10]. Important factors in the activation of *Salmonella* spp. biofilms are the c-di-GMP that is behaving like a secondary messenger molecule when the CsgD content is elevated [34].

Microcolonies are formed by cell proliferation, and many genes involved in cell division, cell wall biogenesis, virulence and motility, stress response, and transcriptional regulation factors are expressed.

Table 1 shows a selection of the genes that are expressed in all the steps of biofilm formation or are upregulated under influence of different biotic or abiotic factors. It was reported that the $\Delta dltABC$ *L. monocytogenes* strains are defective in biofilm formation, validating by transposon mutagenesis, the critical role of D-alanylation of teichoic acids, for biofilm synthesis [24]. So, the mutants without D-alanine on the surface of teichoic acids have a higher negative charge and develop a biofilm-negative phenotype.

The mature biofilm evolves from microcolonies and this development is associated with EPS production. The biofilm matrix of *B. cereus* is similar to other *Bacillus* sp., but the *eps* genes, responsible for the EPS synthesis, are not mandatory for *B. cereus* compared to *B. subtilis* [35]. Little is known about the regulatory networks in *B. cereus*, but studies have shown that CodY and SpoOA may as well play a crucial role in biofilm formation [36].

Furthermore, the structural proteins encoded by *tapA* and *bslA* from *B. subtilis* genome are absent in the matrix of *B. cereus* because these genes have no paralog in *B. cereus* genome. Instead the *tasA* gene is essential for *B. cereus* biofilm development, being responsible for the matrix fiber synthesis [37].

An important polysaccharide identified in the matrix biofilm of many pathogenic bacteria is the colanic acid, which plays an important physiological role for bacteria living in biofilm. This EPS is synthesized by specific enzymes encoded by *wcaL* gene (*S. enterica*) or *wcaF* (*E. coli*). It has been also shown that *rpoS* gene,

the main regulator of the general stress response, may be seen as a key factor in the development of mature biofilms in *E. coli* [38].

Consequently, the transition from the planktonic state to the biofilm state is critical and it is subjected to a strict gene regulation, essential for matrix synthesis, cell aggregation, and cell signaling.

Nevertheless, bacteria of multiple genetic backgrounds communicate by regulating their relationship of cooperativeness through a mechanism called quorum sensing (QS) in which the bacterial cells are having social interactions with each other through small diffusible signal molecules called autoinducers, thus contributing to the biofilm development [10].

Quorum sensing process described in the 1970s is involved in the control of various gene expressions through chemical signaling molecules that are synthesized in response to cell population density [39]. When bacteria start to sense their critical biomass, they answer by activating or repressing genes from 10% of bacteria genome [40]. The system has been described for both Gram-negative and Gram-positive bacteria.

Among QS, other two important regulators are known to control biofilm shape and structure: cyclic diguanosine-5'-monophosphate (c-di-GMP) and small RNAs. For example, *S. aureus* biofilm development is regulated by many environmental conditions and genetic signals. A significant constituent in biofilm formation is mediated by the polysaccharide intercellular adhesin composed mainly of polymeric N-acetyl-glucosamine (PNAG) and eDNA, encoded by the *ica* operon [41]. In certain cases, such as *S. aureus*, biofilm-associated protein (Bap) is involved in biofilm maturation rather than polysaccharide intercellular adhesion (polysaccharide intercellular adhesins) expression [42].

The c-di-GMP involvement in *S. aureus* is an important biofilm regulator that allosterically switches on enzymes of exopolysaccharide biosynthesis [43], while the function of small RNA genes involved is still not yet studied in detail [44]. Although it has been noticed to show an increased susceptibility to disinfectants in planktonic state, however, in biofilm state, it may be among the most resistant ones equally important for food as well as for the medical sectors.

Gram-positive bacteria such as *S. aureus*, *B. subtilis*, and *L. monocytogenes* are communicating through inducers encoded by accessory gene regulator (*Agr*) system (**Table 1**). It seems like the *Agr* complex regulates more than 100 genes in the *S. aureus* genome [45], and its deletion from *L. monocytogenes* genome affects more than 600 genes [46].

The accessory gene regulator of *S. aureus* modulates the expression of virulence factors and toxins in response to autoinducing peptides (AIPs) while *luxS* synthesizes AI-2, which inhibits exopolysaccharide synthesis through an unknown QS cascade [47].

For *S. enterica* and *E. coli*, the QS system is mediated by two genes, *luxS* and *luxR*, homolog to *SdiA* in order to reach intercellular signaling [48, 49].

The *L. monocytogenes* QS signaling triggers the transcriptional activation of one of the virulence PrfA-regulated genes *actA*, resulting in the bacterial aggregation and biofilm formation [10]. Another gene involved in the cell-to-cell interactions is *secA2* gene. Its deletion may inactivate the SecA2 pathway with an increased cell aggregation and sedimentation [50].

4. Fighting against biofilms with nonconventional methods

Since biofilms act as a barrier that protects the embedded cells against cleaning and disinfecting agents [51], the control of biofilm is an issue that is currently

addressed to find effective solutions that can prevent biofilm formation or eliminate the already formed one. Biocontrol of biofilms by using bacteriocins, disruptive enzymes, essential oils, or bacteriophages is gaining importance, as well as using nanoemulsions and nanoparticles. These new methods are promising strategies with remarkable results in the fight against biofilms.

4.1 Bacteriocins used to control biofilms

Bacteriocins are antimicrobial peptides ribosomally produced by an extensive range of bacteria to inhibit or kill competing microorganisms in a micro-ecological system [52, 53]. The most studied bacteriocin and the only one allowed presently as food-grade additive is nisin, a lantibiotic with proven effects against many Gram-positive bacteria including foodborne pathogens [54]. This bacteriocin was shown to penetrate the biofilm formed by *S. aureus* and permeate the sessile bacterial cells by real-time monitoring [55]. Moreover, nisin and its bioengineered derivatives were able to enhance the capability of conventional antibiotics such as chloramphenicol of decreasing *S. aureus* biofilm viability [56]. Nevertheless, a study assessing the effect of neutral electrolyzed water and nisin and their combination against listerial biofilm on glass and stainless steel surfaces indicated the potency of this bacteriocin to improve the efficacy of sanitizers used in food industry [57]. Nisin was also indicated to be effective against biofilms formed by Gram-negative bacteria such as *Salmonella typhimurium* when combined with P22 phage and EDTA, a synergistic combination that reduced 70% of the mature biofilm [58].

Another way to prevent biofilms development is represented by the adsorption of these bioactive compounds on the surfaces that come into contact with foods [59]. In this case, Nisaplin adsorbed to three types of food-contact surfaces commonly encountered in food processing plants, namely stainless steel, polyethylene terephthalate (PET), and rubber, reduced the adhesion ability of food-isolated *L. monocytogenes* strains [60]. Other studies showing the efficacy of nisin in preventing surface colonization by *L. monocytogenes* were conducted by Daeschel et al. [61] and Bower et al. [62].

A bacteriocin found to markedly inhibit the biofilm formed by *S. aureus* is sonorensis, a member of the heterocycloanthracin subfamily produced by *Bacillus sonorensis* MT93 [63].

4.2 Disruptive enzymes for fighting against biofilms

Disruptive enzymes, such as proteases, glycosidases, amylases, cellulases, or DNases, are considered a green alternative to chemical treatments often used in the fight against biofilms' formation in food-related environments [2]. Such enzymes do not have toxic effects and are used both alone and as part of the industrial detergents' composition to improve their cleaning efficacy [64–66].

Proteases are a class of enzymes that catalyzes the cleavage of proteins' peptide bonds. Although they are produced by all living organisms, microbial proteolytic enzymes are preferred over animal or plant origin proteases. The most commonly used source of bacterial proteases is represented by those produced by the genus *Bacillus* since they have remarkable properties such as tolerance to extreme temperatures, large pH domain, organic solvents, detergents, and oxidizing compounds [67]. Given their low substrate specificity, extracellularly produced proteases were shown to be more effective in degrading organic-based aging biofilms compared to amylases [68]. Combinations of a buffer that contained surfactants and dispersing and chelating agents with serine proteases and polysaccharidases were shown to be efficient in removing the biofilms formed by *B. cereus* and *P. fluorescens*, respectively, on stainless

steel slides by the cleaning-in-place procedure [69]. Purified alkaline proteases from *B. subtilis* were reported to degrade biofilms produced by both *P. mendocina* and *E. coli* within 10 minutes [70]. Mold-origin proteases, such as proteinase K, were proved to be effective agents against biofilms formed by *L. monocytogenes* when used either alone or in combination with other biofilms' inhibitors. In a study, proteinase K was capable of complete dispersion of *L. monocytogenes* biofilms grown for 72 h on both plastic and stainless steel surfaces at concentrations above 25 µg/mL. The same study also emphasized the synergistic effect between DNases and proteinase K regarding *L. monocytogenes*-established biofilm dispersion [71].

Polysaccharide-hydrolyzing enzymes were indicated to remove the biofilms formed by *Staphylococcus* spp. and *Pseudomonas* spp. on steel and polypropylene substrata. However, these enzymes did not exhibit a significant bactericidal effect, so they were combined with oxidoreductases for an improved performance [72]. Experimental studies showed that cellulase in conjunction with cetyltrimethylammonium bromide had the capacity of removing 100% of the *S. enterica* mature biofilm at the phase of irreversible attachment. This finding suggests an alternative strategy for removing *Salmonella* biofilms in meat processing facilities [73].

4.3 Using essential oils against biofilms

Plant essential oils (EOs) are rich in phytochemical compounds, which are secondary metabolites produced by plants as defense mechanism against pathogens [74]. Regarding microbial inactivation, EOs have been reported to mainly affect the cellular membrane by permeabilization [75]. This leads to the disruption of vital cellular processes, including energy production, membrane transport, and metabolic regulatory functions [76].

Studies evaluating the potential of EOs as disinfectants were conducted. Leonard et al. [77] assessed the bioactivity of *Syzygium aromaticum* (clove), *Mentha spicata* (spearmint), *Lippia rehmannii*, *Cymbopogon citratus* (lemongrass) EOs, and their major components on the listerial biofilm. The assessment revealed that *M. spicata* and *S. aromaticum* EOs inhibited the growth of listerial biofilm, while, surprisingly, in the presence of their main compounds alone, namely R-(–) carvone and eugenol, respectively, the biofilm biomass increased. Similar phenomenon was previously noticed by [78] in the case of α-pinene, 1,8-cineole, (+)-limonene, linalool, and geranyl acetate, with researchers arguing that bacterial cells in biofilms have a reduced metabolic activity, which make them more resistant to deleterious agents. These results suggest that antimicrobial activity of EOs is rather due to the synergism among the chemical substances that compose them, than due to an individual component's activity. On the other hand, a disinfectant solution based on *Cymbopogon citratus* and *Cymbopogon nardus* EOs was reported to completely reduce the number of *L. monocytogenes* stainless steel surface-adhered cells residing in a 240 h biofilm after 60 min of interaction [79].

Thyme EO has proven antimicrobial properties [80]. In terms of biofilm inhibition capacity, this EO was shown to inhibit significantly the biofilm formed by *B. cereus* [81] and biofilms formed by other food-related pathogens, including *S. aureus* and *E. coli* [82, 83]. Thymol and carvacrol are principal constituents of thyme oil [84], and their potential regarding biofilm inhibition is intensively studied. Surfactant-encapsulated carvacrol was effective against biofilms produced by *E. coli* O157:H7 and *L. monocytogenes* on stainless steel coupons [85]. This natural biocide was also shown to control a dual-species biofilm formed by *S. aureus* and *S. enterica* at quasi-steady state [86]. However, scientists emphasized that carvacrol concentration should be seriously considered when used to combat strong biofilm producers, such as *S. aureus* strains isolated from food-contact surfaces, since low

concentrations may exhibit an inductive effect. In the case of the biofilm formed by *Salmonella* typhimurium on stainless steel surfaces, exposure to thymol resulted in a more pronounced decrease in the biofilm mass compared to exposure to carvacrol or eugenol [87]. Moreover, these compounds enhanced the susceptibility of this pathogen to the treatments with antibiotics such as nalidixic acid [88].

Eugenol is a phytochemical compound preponderantly found in aromatic plants [89]. Interestingly, a study showed that this substance was able to inhibit the intracellular signaling pathway called quorum sensing in the case of biofilms formed by methicillin-resistant *S. aureus* strains isolated from food handlers. This mechanism has an important role in the host colonization, biofilm development, and defense strategies against harmful agents, allowing bacterial cells to act as social communities [90]. EO of bay, clove, pimento berry, and their major constituent, eugenol, were proved to inhibit significantly the biofilm formed by *E. coli* O157:H7. The antibiofilm activity was assigned to the benzene ring of eugenol. Moreover, eugenol led to the downregulation of genes associated with the biofilm formation, attachment, and effacement phenotype, such as curli, fimbriae, and toxin genes [91].

4.4 Fighting against biofilms with bacteriophages

Bacteriophages are viruses that infect bacterial cells. They use the genetic machinery of their host cells to replicate, killing bacteria when reaching a sufficiently high number to produce lysis [92]. They are abundantly encountered anywhere host bacteria live [93] and, therefore, their potential is presently harnessed as natural antimicrobial agents to control pathogenic bacteria in food products and food-related environments [94]. One of the bacteriophages' applications that is intensively explored targets biofilm-forming bacteria that are relevant for food industry, including *L. monocytogenes*, *S. aureus*, *E. coli*, *B. cereus*, and *S. enterica*. However, the success of this approach in fighting biofilms depends on a series of factors such as composition and structure of biofilms, biofilms' maturity, and physiological state of bacterial host residing within biofilms, concentration of bacterial host, or extracellular matrix [95].

Although it is generally thought that biofilms confer resistance to bacteriophages, these bacterial predators developed several mechanisms to destroy bacteria communities. Once they reach the EPS (extracellular polymeric substances) producing host, they start to replicate, resulting in an increased number and, implicitly, in a progressive degradation of the biofilms and prevention of their regeneration. Bacteriophages can also express or induce the expression from within host genome of depolymerizing enzymes that degrade EPS. Nevertheless, they can also infect persister cells, which are dormant variants of regular bacterial cells that are highly resistant to antibiotics. In this case, the lysis process is triggered once persister bacteria are reactivated [96].

Scientists [97] reported the ability of a bred phage to reduce L-form biofilms formed by *L. monocytogenes* on stainless steel surfaces. This bacteriophage was as effective as lactic acid (130 ppm) in the eradication of preformed L-form biofilms. P100 phage treatment was also shown to reduce the number of *L. monocytogenes* cells under biofilm conditions on stainless steel coupon surface regardless of serotype [98]. The potency of three bacteriophages, namely LiMN4L, LiMN4p, and LiMN17, used as a cocktail or individually at $\sim 9 \log_{10}$ PFU/mL was evaluated to inactivate *L. monocytogenes* cells residing within 7-day biofilms strongly adhered to clean or fish broth-coated stainless steel coupons and dislodged biofilm cells [99]. These phages exhibited a higher efficiency in the case of dislodged cells compared to intact biofilms when applied for short periods of time. Therefore, for high efficiency, short-term phage treatments in fish processing environments may require

prior processes aiming at disrupting the biofilms [99]. The ability of *Salmonella* spp. to develop biofilms was shown to depend on the attachment surface types that may be encountered in chicken slaughterhouses. With regard to this, surfaces such as glass and stainless steel favored the formation of *Salmonella* biofilms, while polyvinyl chloride surface sustained less the development of them. The antibiofilm activity of a pool of bacteriophages isolated from hospital and poultry wastewater was concentrated at 3 h of action for all types of surfaces. Curiously, biofilms attached to the glass surface were resistant to a 6-h treatment. Bacteriophages were able to degrade the glass-attached biofilms after 9 h of interaction [100]. A bacteriophage BPECO 19 was evaluated as possible inhibitor of a three *E. coli* O157:H7 strain biofilm grown on both abiotic (stainless steel, rubber, and minimum biofilm eradication concentration device) and biotic (lettuce leaves) surfaces. This bacteriophage showed great biofilm inhibition activity on all the tested surfaces, being suggested as effective antibiofilm agent in food industry [101].

4.5 Nanotechnology-based antimicrobials used to control biofilms

Currently, controlling biofilm formation by nanotechnology-based antimicrobials is of industrial interest, nanoemulsions and nanoparticles (NPs) with antibiofilm activity being an alternative to conventional methods.

Recently, some studies made on model system (polystyrene well plates) and real systems (fresh pineapple, tofu, and lettuce) indicated that nanoemulsions of EOs have significantly higher antibiofilm activity compared to pure EOs (Table 2). Antimicrobial efficacy of nanoemulsions is dependent on the droplet size and electrical properties of nanoemulsions [102, 103], nature of bacteria [75, 104], and food matrix [105–107].

Nanoparticles (NPs) can be used for both inhibition of biofilm formation and eradication of already formed ones [108].

In the last period, NPs with natural compounds gained increased interest because it was demonstrated that the inorganic capsules can protect the natural products with antimicrobial activity [109]. In this respect, cinnamaldehyde-encapsulated chitosan nanoparticles, garlic-silver NPs, and “tree of tee” oil NPs were used to combat biofilm formation by *P. aeruginosa* on polystyrene well plates and glass pieces [110–112]. Meanwhile, the biofilm formed by *S. aureus* on glass slide was inhibited by applying gold NPs with EO of *Nigella sativa* [113] and garlic-silver NPs [111].

Nanoemulsion	Particle size, nm	Biofilm-forming bacteria	Mode of action	Ref.
EO of <i>Citrus medica</i> L. var. <i>sarcodactylis</i>	73	<i>S. aureus</i>	Inhibit the ability of bacteria to attach to surfaces	[185]
EO of <i>Cymbopogon flexuosus</i> (lemongrass)	78.46 ± 0.51	<i>P. aeruginosa</i> (PA01) and <i>S. aureus</i> (ATCC 29213)	Reduce the adhesion of pathogenic bacteria to surfaces	[186]
Trans-CA	>100 <100	<i>P. aeruginosa</i> (CMCC 10104), <i>S. typhimurium</i> and <i>S. aureus</i>	Membrane disruption by destabilization of lipids	[187]
Linalool	10.9 ± 0.1	<i>S. typhimurium</i> (ATCC 1331)	Cell membrane integrity disruption	[107]

Table 2.
Antibiofilm activity of essential oil (EO) nanoemulsions.

Metal-based NPs (silver, gold, and metal oxides) with antimicrobial activity can be used to create different nanocomposite materials able to prevent bacterial adhesiveness to food-contact surfaces and equipment. Wu and coworkers [114] showed that cysteine dithiothreitol and beta-mercaptoethanol were able to reduce *S. aureus* biofilm formation on polystyrene polymer. Liang and coworkers [115] revealed that silver salt of 12-tungstophosphoric acid NPs (AgWPA-NPs) can be used to develop new materials for preserving foods, since they were able to inhibit *S. aureus* biofilm formation by damaging bacterial cells' membrane. Moreover, genes related to biofilm formation, such as *icaA*, *sarA*, and *cidA* were shown to be downregulated as a consequence of AgWPA-NPs' application. Naskar and coworkers [116] tested the antibiofilm activity of polyethylene glycol-coupled Ag-ZnO-rGO (AZGP) nanocomposite on both Gram-positive bacteria (*S. aureus* ATCC 25923) and Gram-negative bacteria (*P. aeruginosa* MTCC 2453). These NPs, at a concentration of 31.25 µg/mL, reduced the biofilm formed by *S. aureus* with ~95% and that formed by *P. aeruginosa* with ~93%. Zinc oxide NPs were used for the destruction of the biofilm formed on glass slide by *S. aureus* and *P. aeruginosa* [117]. Titania nanoparticles can be used to prevent the formation of *P. fluorescens* biofilm on the surfaces of TiO₂/polystyrene nanocomposite film [118]. It has been shown that nanostructured TiO₂ combined with UVA irradiation can be used to destroy *L. monocytogenes* biofilm, while silver NPs at a concentration of 15 µg/mL had the capacity to inhibit *S. aureus* and *E. coli* biofilms [119, 120].

The ability of two types of superparamagnetic iron oxide (IONs and IONs coated with 3-aminopropyltriethoxysilane) to inhibit biofilm formation by *B. subtilis* was successfully tested by [121].

5. Food technologies to control the biofilm formation

Some food technologies belonging to alternative technologies seem to be successful for preventing the biofilm formation and/or for targeting resistant microorganisms and making them more susceptible to molecular interventions in order to hinder their biofilm formation ability. Among these technologies are included plasma treatments, ultrasound treatments, light-based technologies, pulsed electric fields (PEF), and high hydrostatic pressures. With the exception of ultrasound treatments that can be used to fight against biofilms formed on mechanical parts or pipes, the others are mostly applied for food matrix decontamination.

5.1 Plasma treatments

Plasma is generated when the added energy ionizes a gas, which is composed of ions, neutrals, and electrons. Plasma treatment is a surface treatment that has a low penetration depth and was reported to be effective against biofilms, depending on the type of surface biofilms are formed on, the distance between plasma and surface, and the thickness or the microbial load.

Plasma sources for producing nonthermal plasma at atmospheric pressure are plasma jets, dielectric barrier discharges (DBD), corona discharges, and microwave discharges. Different other characteristics of the plasma have been reported to influence the biofilms' inactivation such as the setup (electrode configuration), the exposure mode, the operating gas, the frequency, the plasma intensity (voltage), and the time of exposure [122].

Researches [123] showed that the efficacy of DBD in-package atmospheric cold plasma (ACP) against *S. typhimurium*, *L. monocytogenes*, and *E. coli* could reach up to 5 log CFU/g after 300 s of treatment at 80 kV. Other researchers [124] studied the

effect of ACP on monoculture biofilms (*E. coli*, *S. enterica*, *L. monocytogenes*, and *P. fluorescens*) and mixed culture biofilms (*L. monocytogenes* and *P. fluorescens*) and demonstrated that the latest are more difficult to inactivate than the former ones. *L. monocytogenes* and *P. fluorescens* inoculated as mixed cultures on lettuce were reduced by 2.2 and 4 log CFU/g, respectively, and the biofilms formed at 4°C were more resistant than the ones formed at 15°C.

Govaert et al. [122] studied the influence of different plasma characteristics on the inactivation of *L. monocytogenes* and *S. typhimurium* biofilms and showed that inactivation can vary from 1 log to approximately 3.5 log (CFU/cm²), but the highest reduction was obtained for a DBD electrode with He and no O₂ in the gas mixture and an input voltage of 21.88 V. A high efficiency of the inactivation of bacterial biofilm was achieved by DBD for low-dose discharges (70 mW/cm²) and short treatment times (≤300 s), and the most effective reduction in the number of *S. aureus* cells of 2.77 log was reported after 300 s. *E. coli* biofilm was reduced only by 66.7% [125].

It was shown that ACP is a promising technique but alone cannot achieve complete biofilm inactivation and thus it should be complemented by other surface treatments. Possibility to combine ACP with different biocides such as hydrogen peroxide, sodium hypochlorite, ethylenediaminetetraacetic acid, chlorhexidine, octenidine, and polyhexanide applied before or after the plasma treatment was tested by [126] to reduce biofilms cultivated on titanium discs. Also, Gupta et al. [127] studied the antimicrobial effect of an ACP, plasma jet combined with chlorhexidine, for the sterilization of the biofilms formed by *P. aeruginosa* on titanium surfaces [128].

5.2 Ultrasound-assisted technologies

Ultrasound (US) is a form of energy generated by sound waves at frequencies that are too high to be detected by the human hearing (>16 kHz). The US band is also divided into low frequency (16 kHz–1 MHz) and high frequency (>1 MHz) bands.

US was used as biofilm removal method; however, many studies demonstrated that it should be complemented by other inactivation methods [129, 130]. For example, [130] demonstrated that US removed a significant amount of *E. coli* and *S. aureus* biofilm, up to 4 times higher compared to the swabbing method. Later on, the same researchers [131] showed that two ultrasonic devices developed failed to completely remove *E. coli* and *S. aureus* biofilms for closed surfaces, but they succeeded in biofilm inactivation on opened surfaces (10 s at 40 kHz). The use of chelating agents such as EDTA completely dislodged *E. coli* biofilm but not significantly improved *S. aureus* biofilm removal. A synergistic effect was achieved when US was combined with enzymes (proteolytic or glycolytic) that demonstrated a 2–3 times higher efficacy in biofilm removal compared to sonication.

Combination of US with mild heat and slightly acidic electrolyzed water was used to test the inactivation of *B. cereus* biofilms on green leaf surfaces. Slightly acidic electrolyzed water with 80 mg/L treatment for 15 min combined with US of fixed frequency (40 kHz) and acoustic energy density of 400 W/l at 60°C resulted in a reduction of ~3.0 and ~3.4 log CFU/cm² of *B. cereus* reference strains ATCC 10987 and ATCC 14579 [132].

Synergistic effects were registered also for ultrasound (US; 37 kHz, 380 W for 10–60 min) assisted by peroxyacetic acid (PAA; 50–200 ppm) on reducing *Cronobacter sakazakii* biofilms on cucumbers [133].

The efficacy of US (37 kHz, 200 W, for 30 min)-assisted chemical cleaning methods (10% alcohols, 2.5% benzalkonium chloride, and 2.5% didecyl dimethyl

ammonium chloride) for the removal of *B. cereus* biofilm from polyurethane conveyor belts in bakeries using US was better compared to each individual method as demonstrated by [134].

5.3 Combined light-based technologies

Ultraviolet (UV) light technology is based on the emission of radiation within the ultraviolet region (100–400 nm). The antimicrobial behavior of UV light is based on the formation of DNA photoproducts that inhibit transcription and replication and can lead to cell death [135]. Since the absorption of the DNA is in the 200–280 nm range with the maximum at 254 nm, this wavelength of the UV-C range is called germicidal UV light [136].

Pulsed light (PL) is the next-generation approach to UV delivery. PL is a technology that can be used to decontaminate surfaces by generating short-time high-energy light pulses (millions or thousands of a second) of an intense broad spectrum (200–1100 nm). PL can be used to decontaminate a great variety of foods as well as to decontaminate contact surfaces, thus improving safety in foods and extending their shelf life [137]. The antimicrobial effect is based on strand breaks that lead to the destruction/chemical modification of the DNA and thus prevent the replication of the bacterial cell [138].

Recently, Rajkovic and coworkers [139] evaluated the efficacy of pulsed UV light treatments to reduce *S. typhimurium*, *E. coli* O157:H7, *L. monocytogenes*, and *S. aureus* on the surface of dry fermented salami inoculated with 6.3 log CFU/g at 3 J/cm² (1 pulse) or 15 J/cm² (5 pulses) for 1 or 30 min. The authors found a significant effect of PL treatment time, with the best results after 1 min of applying PL (2.18–2.42 log CFU/g reduction), while after 30 min, the reduction varied from 1.14 to 1.46 log CFU/g.

A comprehensive review in the literature underlined the various researches directed mainly at inactivation of pathogens in food or on surfaces and for preventing biofilm formation [137]. While there are often considerable differences in the rate of microbial inactivation by PL, a maximum reduction of 3-log was typically achieved, which is below the reduction performance standard of 5-log required by HACCP regulation [138].

Regarding the combined methods, synergistic interaction between gallic acid and UV-A light was able to inactivate *E. coli* O157:H7 in spinach biofilm [140]. The UV-A treatment complemented by the gallic acid presence was found to be effective producing a 3-log (CFU/mL) reduction in *E. coli* O157:H7 on the surface of spinach leaves.

However, PL technology limitation related to the inability to effectively treat uneven food surfaces with crevices, the presence of organic material, and large microbial populations generating shading effects should also be taken into account. Future innovation in PL technology will seek to improve fluence efficiency, for example by considering alternative light sources such as LEDs [141], reflective surfaces included in the treatment chamber, using materials such as titanium dioxide to augment irradiation efficacy [138], and other combination of treatments assisted by PL, based on hurdle approach.

5.4 Pulsed electric field

Pulsed electric field (PEF) is a food processing technology that applies short, high-voltage pulses, across a food material placed between two or more electrodes. The pulses enhance cell permeability by damaging the cell membrane, and if the transmembrane potential is sufficiently high, it produces electroporation. Further, if pores are not resealed, it results in cell death. Most of the food applications are

designed for liquid flow through pipes where in a certain region the liquid passes in-between the electrodes area that applies the PEFs [142].

Thermosonication (TS) was investigated in combination with PEF to determine its effects on inactivation and sublethal injury of *P. fluorescens* and *E. coli*. While TS was applied at a low (18.6 mm) and high (27.9 mm) wave amplitude, PEF was applied at a low (29 kV cm⁻¹) and high electrical field strength (32 kV cm⁻¹). TS/PEF caused a maximum of 66% inactivation, while sublethally injuring approximately 26% of the *E. coli* population [143].

PEF demonstrated synergistic potential in combination with additives (EDTA or triethyl citrate) to inactivate *Salmonella* serovars in whole liquid eggs [144].

There is a lot of potential demonstrated by PEF and the combination with different other hurdles could contribute to the elimination of persistent clones able to form biofilms.

5.5 High pressure processing

High pressure processing (HPP) is a cutting-edge technology that represents an alternative to conventional processing. HPP has the ability to inactivate microorganisms and enzymes and has a minimal impact on sensorial and nutritional properties of food [145, 146].

Combined with other different hurdles, the pressure-assisted processing could be oriented toward a more targeted inactivation of pathogens and prevention of biofilm formation.

Recent studies were focused on *L. monocytogenes*, a pathogen able to form surface-attached communities that have high tolerance to stress. In order to understand how *agr* gene regulates virulence and biofilm formation, a recent molecular study [147] was conducted. *L. monocytogenes* EGD-e $\Delta agrD$ showed reduced levels of surface-attached biomass in 0.1 BHI (brain heart infusion) broth.

However, *L. monocytogenes* mutant deficient in *agr* peptide sensing showed no impaired resistance to HPP treatment at 200, 300, and 400 MPa for 1 min compared to wild-type and *L. monocytogenes* EGD-e and thus demonstrating that weakened resistance to cell wall stress is not responsible for the reduced biofilm-forming ability.

Understanding better the molecular mechanisms of stress-related genes will allow to better target pathogen inactivation and to select the right hurdle combination and parameters of unconventional technologies to able to reduce the susceptibility of certain pathogens to form biofilms. These types of studies are just at the beginning and many more researches are expected to focus on these topics in the near future.

6. Conclusions

Pathogenic and toxigenic bacteria are able to form biofilms, structures that protect the cells and allow them to remain postsanitation in the food processing environment.

Specific genes are expressed in all the steps of biofilm formation or are upregulated under influence of different biotic or abiotic factors. Genes codify for cell surface structures and appendages (flagella, curli, fimbriae, and pili) that are facilitating biofilm formation by helping bacteria to move toward surfaces and to adhere to them, for extracellular polymeric substances that stabilize the biofilms and protect the cells and for quorum sensing communication.

Scientists developed novel agents and strategies to control biofilm formation or removal. Their application to the food industry would contribute to eradication of undesirable bacteria from food-processing environments and, subsequently, from food products.

Acknowledgements

This study was supported by a grant of the Executive Agency for Higher Education, Research, Development and Innovation Funding in Romania awarded to the Dunarea de Jos University of Galati (International and European Cooperation—Subprogramme 3.2—Horizon 2020—Contract no: 15/2017), an institution that is a member of the ERA-IB2 consortium “SafeFood” (ID: ERA-IB-16-014).

Florentina Ionela Bucur’s work has been funded by the European Social Fund through the Sectoral Operational Programme Human Capital 2014–2020, through the Financial Agreement with the title “Scholarships for entrepreneurial education among doctoral students and postdoctoral researchers (Be Entrepreneur!),” Contract no. 51680/09.07.2019—SMIS code: 124539.

Author details

Leontina Grigore-Gurgu, Florentina Ionela Bucur, Daniela Borda,
Elena-Alexandra Alexa, Corina Neagu and Anca Ioana Nicolau*
Dunarea de Jos University of Galati, Faculty of Food Science and Engineering,
Galati, Romania

*Address all correspondence to: anca.nicolau@ugal.ro

IntechOpen

© 2019 The Author(s). Licensee IntechOpen. This chapter is distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/3.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

References

- [1] Téllez S. Biofilms and their impact on food industry. VISAVET Outreach Journal. 2010. Available from: <https://www.visavet.es/en/articles/biofilms-impact-food-industry.php> [Accessed 1.10.2019]
- [2] Galié S, García-Gutiérrez C, Miguélez EM, Villar CJ, Lombó F. Biofilms in the food industry: Health aspects and control methods. *Frontiers in Microbiology*. 2018;**9**:898. DOI: 10.3389/fmicb.2018.00898
- [3] Coughlan LM, Cotter PD, Hill C, Alvarez-Ordóñez A. New weapons to fight old enemies: Novel strategies for the (bio)control of bacterial biofilms in the food industry. *Frontiers in Microbiology*. 2016;**7**:1641. DOI: 10.3389/fmicb.2016.01641
- [4] Trachoo N. Biofilms and the food industry. *Songklanakarin. Journal of Science and Technology*. 2003;**25**(6):807-815
- [5] Beloin C, Ghigo JM. Finding gene-expression patterns in bacterial biofilms. *Trends in Microbiology*. 2005;**13**:16-19. DOI: 10.1016/j.tim.2004.11.008
- [6] Chmielewski RAN, Frank JF. Biofilm formation and control in food processing facilities. *Comprehensive Reviews in Food Science and Food Safety*. 2006;**2**:22-32. DOI: 10.1111/j.1541-4337.2003.tb00012.x
- [7] Lemon KP, Higgins DE, Kolter R. Flagellar motility is critical for *Listeria monocytogenes* biofilm formation. *Journal of Bacteriology*. 2007;**189**:4418-4424. DOI: 10.1128/JB.01967-06
- [8] Renier S, Hébraud M, Desvaux M. Molecular biology of surface colonization by *Listeria monocytogenes*: An additional facet of a Gram-positive foodborne pathogen. *Environmental Microbiology*. 2011;**13**:835-850. DOI: 10.1111/j.1462-2920.2010.02378.x
- [9] Barnhart MM, Chapman MR. Curli biogenesis and function. *Annual Review of Microbiology*. 2006;**60**:131-147. DOI: 10.1146/annurev.micro.60.080805.142106
- [10] Giaouris E, Heir E, Desvaux M, Hébraud M, Møretrø T, Langsrud S, et al. Intra- and inter-species interactions within biofilms of important foodborne bacterial pathogens. *Frontiers in Microbiology*. 2015;**6**:841. DOI: 10.3389/fmicb.2015.00841
- [11] Vestby LK, Møretrø T, Langsrud S, Heir E, Nesse LL. Biofilm forming abilities of *Salmonella* are correlated with persistence in fish meal and feed factories. *BMC Veterinary Research*. 2009;**5**:20. DOI: 10.1186/1746-6148-5-20
- [12] Van Houdt R, Michiels CW. Role of bacterial cell surface structures in *Escherichia coli* biofilm formation. *Research in Microbiology*. 2005;**156**(5-6):626-633. DOI: 10.1016/j.resmic.2005.02.005
- [13] Mandin P, Guillier M. Expanding control in bacteria: Interplay between small RNAs and transcriptional regulators to control gene expression. *Current Opinion in Microbiology*. 2013;**16**(2):125-132. DOI: 10.1016/j.mib.2012.12.005
- [14] Vogelee P, Tremblay YDN, Jubelin G, Jacques M, Harel J. Biofilm-forming abilities of Shiga toxin-producing *Escherichia coli* isolates associated with human infections. *Applied and Environmental Microbiology*. 2016;**82**(5):1448-1458. DOI: 10.1128/AEM.02983-15
- [15] Alvarez-Ordóñez A, Coughlan LM, Briandet R, Cotter PD. Biofilms in food processing environments: Challenges and opportunities. *Annual Review of Food Science and Technology*.

2019;**10**:10.1-10.23. DOI: 10.1146/annurev-food-032818-121805

[16] Christison CA, Lindsay D, von Holy A. Cleaning and handling implements as potential reservoirs for bacterial contamination of some ready-to-eat foods in retail delicatessen environments. *Journal of Food Protection*. 2007;**70**:2878-2883. DOI: 10.4315/0362-028X-70.12.2878

[17] De Been M, Francke C, Moezelaar R, Abee T, Siezen RJ. Comparative analysis of two-component signal transduction systems of *Bacillus cereus*, *Bacillus thuringiensis* and *Bacillus anthracis*. *Microbiology*. 2006;**152**:3035-3048. DOI: 10.1099/mic.0.29137-0

[18] Houry A, Briandet R, Aymerich S, Gohar M. Involvement of motility and flagella in *Bacillus cereus* biofilm formation. *Microbiology*. 2010;**156**:1009-1018. DOI: 10.1099/mic.0.034827-0

[19] Houry A, Gohar M, Deschamps J, Tischenko E, Aymerich S, Gruss A, et al. Bacterial swimmers that infiltrate and take over the biofilm matrix. *Proceedings of the National Academy of Sciences of the United States of America*. 2012;**109**:13088-13093. DOI: 10.1073/pnas.1200791109

[20] Fratamico PM, Annous BA, Guenther NW. *Biofilms in the Food and Beverage Industries*. Oxford; Cambridge; Philadelphia, New Delhi: Woodhead Publishing Limited; 2012. ISBN: 978-1-84569-716-7

[21] Oniciuc EA, Cerca N, Nicolau AI. Compositional analysis of biofilms formed by *Staphylococcus aureus* isolated from food sources. *Frontiers in Microbiology*. 2016;**7**:390. DOI: 10.3389/fmicb.2016.00390

[22] Xue T, Chen X, Shang F. Short communication: Effects of lactose and milk on the expression of biofilm

associated genes in *Staphylococcus aureus* strains isolated from a dairy cow with mastitis. *Journal of Dairy Science*. 2014;**97**(10):6129-6134. DOI: 10.3168/jds.2014-8344

[23] Tirumalai PS, Prakash S. Expression of virulence genes by *Listeria monocytogenes* J0161 in natural environment. *Brazilian Journal of Microbiology*. 2012;**43**(2):834-843. DOI: 10.1590/S1517-83822012000200050

[24] Alonso AN, Perry KJ, Regeimbal JM, Regan PM, Higgins DE. Identification of *Listeria monocytogenes* determinants required for biofilm formation. *PLoS One*. 2014;**9**(12):e113696. DOI: 10.1371/journal.pone.0113696

[25] Luo Q, Shang J, Feng X, Guo X, Zhang L, Zhou Q. PrfA led to reduced biofilm formation and contributed to altered gene expression patterns in biofilm-forming *Listeria monocytogenes*. *Current Microbiology*. 2013;**67**:372-378. DOI: 10.1007/s00284-013-0377-7

[26] Price R, Jayeola V, Niedermeyer J, Parsons C, Kathariou S. The *Listeria monocytogenes* key virulence determinants hly and prfA are involved in biofilm formation and aggregation but not colonization of fresh produce. *Pathogens*. 2018;**7**(1):E18. DOI: 10.3390/pathogens7010018

[27] Colagiorgi A, Bruini I, Di Ciccio PA, Zanardi E, Ghidini S, Ianieri A. *Listeria monocytogenes* biofilms in the wonderland of food industry. *Pathogens*. 2017;**6**(3):41. DOI: 10.3390/pathogens6030041

[28] Mack D, Fischer W, Krokotsch A, Leopold K, Hartmann R, Egge H, et al. The intercellular adhesin involved in biofilm accumulation of *Staphylococcus epidermidis* is a linear beta-1,6-linked glucosaminoglycan: Purification and structural analysis. *Journal of Bacteriology*. 1996;**178**:175-183. DOI: 10.1128/jb.178.1.175-183.1996

- [29] Resch A, Rosenstein R, Nerz C, Götz F. Differential gene expression profiling of *Staphylococcus aureus* cultivated under biofilm and planktonic conditions. *Applied and Environmental Microbiology*. 2005;**71**(5):2663-2676. DOI: 10.1128/AEM.71.5.2663-2676.2005
- [30] van der Woude MW. Re-examining the role and random nature of phase variation. *FEMS Microbiology Letters*. 2006;**254**:190-197. DOI: 10.1111/j.1574-6968.2005.00038.x
- [31] Ranjith K, Arunasri K, Sathyanarayana Reddy G, Adicherla HK, Sharma S, Shivaji S. Global gene expression in *Escherichia coli*, isolated from the diseased ocular surface of the human eye with a potential to form biofilm. *Gut Pathogens*. 2017;**9**:15. DOI: 10.1186/s13099-017-0164-2
- [32] Salazar JK, Deng D, Tortorello ML, Brandl MT, Wang H, Zhang W. Genes *ycfR*, *sirA* and *yigG* contribute to the surface attachment of *Salmonella enterica* Typhimurium and Saint Paul to fresh produce. *PLoS One*. 2013;**8**(2):e57272. DOI: 10.1371/journal.pone.0057272
- [33] Chin KCJ, Duane T, Hebrard TM, Anbalagan K, Dashti MG, Phua KK. Transcriptomic study of *Salmonella enterica* subspecies *enterica* serovar Typhi biofilm. *BMC Genomics*. 2017;**18**:836. DOI: 10.1186/s12864-017-4212-6
- [34] MacKenzie KD, Palmer MB, Köster WL, White AP. Examining the link between biofilm formation and the ability of pathogenic *Salmonella* strains to colonize multiple host species. *Frontiers in Veterinary Science*. 2017;**4**:138. DOI: 10.3389/fvets.2017.00138
- [35] Gao T, Foulston L, Chai Y, Wang Q, Losick R. Alternative modes of biofilm formation by plant-associated *Bacillus cereus*. *Microbiology Open*. 2015;**4**:452-464. DOI: 10.1002/mbo3.251
- [36] Majed R, Faille C, Kallassy M, Gohar M. *Bacillus cereus* biofilms—Same, only different. *Frontiers in Microbiology*. 2016;**7**:1054. DOI: 10.3389/fmicb.2016.01054
- [37] Caro-Astorga J, Pérez-García A, De Vicente A, Romero D. A genomic region involved in the formation of adhesin fibers in *Bacillus cereus* biofilms. *Frontiers in Microbiology*. 2015;**5**:745. DOI: 10.3389/fmicb.2014.00745
- [38] Álvarez-Ordóñez A, Alvseike O, Omer MK, Heir E, Axelsson L, Holck A, et al. Heterogeneity in resistance to food-related stresses and biofilm formation ability among verocytotoxigenic *Escherichia coli* strains. *International Journal of Food Microbiology*. 2013;**161**(3):220-230. DOI: 10.1016/j.ijfoodmicro.2012.12.008
- [39] Schauder S, Bassler BL. The languages of bacteria. *Genes and Development*. 2001;**15**(12):1468-1480. DOI: 10.1101/gad.899601
- [40] Zhao L, Xue T, Shang F, Sun H, Sun B. *Staphylococcus aureus* AI-2 quorum sensing associates with the KdpDE two-component system to regulate capsular polysaccharide synthesis and virulence. *Infection and Immunity*. 2010;**78**(8):3506-3515. DOI: 10.1128/IAI.00131-10
- [41] Fluckiger U, Ulrich M, Steinhuber A, Döring G, Mack D, Landmann R, et al. Biofilm formation, *icaADBC* transcription, and polysaccharide intercellular adhesin synthesis by staphylococci in a device-related infection model. *Infection and Immunity*. 2005;**73**(3):1811-1819. DOI: 10.1128/IAI.73.3.1811-1819.2005
- [42] Speziale P, Pietrocola G, Foster TJ, Geoghegan JA. Protein-based biofilm matrices in staphylococci. *Frontiers in Cellular and Infection Microbiology*. 2014;**4**:171. DOI: 10.3389/fcimb.2014.00171

- [43] Holland LM, O'Donnell ST, Ryjenkov DA, Gomelsky L, Slater SR, Fey PD, et al. A staphylococcal GGDEF domain protein regulates biofilm formation independently of cyclic dimeric GMP. *Journal of Bacteriology*. 2008;**190**:5178-5189. DOI: 10.1128/JB.00375-08
- [44] Romilly C, Caldelari I, Parmentier D, Lioliou E, Romby P, Fechter P. Current knowledge on regulatory RNAs and their machineries in *Staphylococcus aureus*. *RNA Biology*. 2012;**9**:402-413. DOI: 10.4161/rna.20103
- [45] Fujii T, Ingham C, Nakayama J, Beerthuyzen M, Kunuki R, Molenaar D, et al. Two homologous Agr-like quorum-sensing systems cooperatively control adherence, cell morphology, and cell viability properties in *Lactobacillus plantarum* WCFS1. *Journal of Bacteriology*. 2008;**190**:7655-7665. DOI: 10.1128/JB.01489-07
- [46] Riedel CU, Monk IR, Casey PG, Waidmann MS, Gahan CGM, Hill C. AgrD-dependent quorum sensing affects biofilm formation, invasion, virulence and global gene expression profiles in *Listeria monocytogenes*. *Molecular Microbiology*. 2009;**71**:1177-1189. DOI: 10.1111/j.1365-2958.2008.06589.x
- [47] Wolska KI, Grudniak AM, Rudnicka Z, Markowska K. Genetic control of bacterial biofilms. *Journal of Applied Genetics*. 2016;**57**:225-238. DOI: 10.1007/s13353-015-0309-2
- [48] Surette MG, Miller MB, Bassler BL. Quorum sensing in *Escherichia coli*, *Salmonella Typhimurium*, and *Vibrio harveyi*: A new family of genes responsible for autoinducer production. *Proceeding of the National Academy of Sciences of the USA*. 1999;**96**:1639-1644. DOI: 10.1073/pnas.96.4.1639
- [49] Challan Belval S, Gal L, Margiewes S, Garmyn D, Piveteau P, Guzzo J. Assessment of the roles of LuxS, S-ribosyl homocysteine, and autoinducer 2 in cell attachment during biofilm formation by *Listeria monocytogenes* EGD-e. *Applied and Environmental Microbiology*. 2006;**72**:2644-2650. DOI: 10.1128/AEM.72.4.2644-2650.2006
- [50] Renier S, Chagnot C, Deschamps J, Caccia N, Szlavik J, Joyce SA. Inactivation of the SecA2 protein export pathway in *Listeria monocytogenes* promotes cell aggregation, impacts biofilm architecture and induces biofilm formation in environmental conditions. *Environmental Microbiology*. 2014;**16**:1176-1192. DOI: 10.1111/1462-2920.12257
- [51] Flemming HC, Wingender J, Szewzyk U, Steinberg P, Rice SA, Kjelleberg S. Biofilms: An emergent form of bacterial life. *Nature Reviews Microbiology*. 2016;**14**:563-575. DOI: 10.1038/nrmicro.2016.94
- [52] Zacharof MP, Lovitt RW. Bacteriocins produced by lactic acid bacteria a review article. *APCBEE Procedia*. 2012;**2**:50-56. DOI: 10.1016/j.apcbee.2012.06.010
- [53] Riley MA, Wertz JE. Bacteriocins: Evolution, ecology, and application. *Annual Review of Microbiology*. 2002;**56**:117-137. DOI: 10.1146/annurev.micro.56.012302.16
- [54] de Arauz LJ, Jozala AF, Mazzola PG, Vessoni Penna TC. Nisin biotechnological production and application: A review. *Trends in Food Science & Technology*. 2009;**20**:146-154. DOI: 10.1016/j.tifs.2009.01.056
- [55] Godoy-Santos F, Pitts B, Stewart PS, Mantovani HC. Nisin penetration and efficacy against *Staphylococcus aureus* biofilms under continuous-flow conditions. *Microbiology*. 2019;**165**:761-771. DOI: 10.1099/mic.0.000804

- [56] Field D, O'Connor R, Cotter PD, Ross RP, Hill C. *In vitro* activities of nisin and nisin derivatives alone and in combination with antibiotics against *Staphylococcus* biofilms. *Frontiers in Microbiology*. 2016;7:1-11. DOI: 10.3389/fmicb.2016.00508
- [57] Arevalos-Sánchez M, Regalado C, Martin SE, Domínguez-Domínguez J, García-Almendárez BE. Effect of neutral electrolyzed water and nisin on *Listeria monocytogenes* biofilms, and on listeriolysin O activity. *Food Control*. 2012;24:116-122. DOI: 10.1016/j.foodcont.2011.09.012
- [58] Yüksel FN, Buzrul S, Akçelik M, Akçelik N. Inhibition and eradication of *Salmonella* Typhimurium biofilm using P22 bacteriophage, EDTA and nisin. *Biofouling*. 2018;34:1046-1054. DOI: 10.1080/08927014.2018.1538412
- [59] Kumar CG, Anand SK. Significance of microbial biofilms in food industry: A review. *International Journal of Food Microbiology*. 1998;42:9-27. DOI: 10.1016/s0168-1605(98)00060-9
- [60] Guerra NP, Araujo AB, Barrera AM, Agrasar AT, Macías CL, Carballo J, et al. Antimicrobial activity of nisin adsorbed to surfaces commonly used in the food industry. *Journal of Food Protection*. 2005;68:1012-1019. DOI: 10.4315/0362-028x-68.5.1012
- [61] Daeschel MA, McGuire J, Al-Makhlafi H. Antimicrobial activity of nisin adsorbed to hydrophilic and hydrophobic silicon surfaces. *Journal of Food Protection*. 1992;55:731-735. DOI: 10.4315/0362-028x-55.9.731
- [62] Bower CK, McGuire J, Daeschel MA. Suppression of *Listeria monocytogenes* colonization following adsorption of nisin onto silica surfaces. *Applied and Environmental Microbiology*. 1995;61:992-997
- [63] Chopra L, Singh G, Kumar Jena K, Sahoo DK. Sonorensin: A new bacteriocin with potential of an anti-biofilm agent and a food biopreservative. *Scientific Reports*. 2015;5:13412. DOI: 10.1038/srep13412
- [64] Stiefel P, Mauerhofer S, Schneider J, Maniura-Weber K, Rosenberg U, Ren Q. Enzymes enhance biofilm removal efficiency of cleaners. *Antimicrobial Agents and Chemotherapy*. 2016;60:3647-3652. DOI: 10.1128/AAC.00400-16
- [65] Liu X, Tang B, Gu Q, Yu X. Elimination of the formation of biofilm in industrial pipes using enzyme cleaning technique. *MethodsX*. 2014;1:130-136. DOI: 10.1016/j.mex.2014.08.008
- [66] Torres CE, Lenon G, Craperi D, Wilting R, Blanco Á. Enzymatic treatment for preventing biofilm formation in the paper industry. *Applied Microbiology and Biotechnology*. 2011;92:95-103. DOI: 10.1007/s00253-011-3305-4
- [67] Contesini FJ, de Melo RR, Sato HH. An overview of *Bacillus* proteases: From production to application. *Critical Reviews in Biotechnology*. 2017;38:321-334. DOI: 10.1080/07388551.2017.1354354
- [68] Huang H, Ren H, Ding L, Geng J, Xu K, Zhang Y. Aging biofilm from a full-scale moving bed biofilm reactor: Characterization and enzymatic treatment study. *Bioresource Technology*. 2014;154:122-130. DOI: 10.1016/j.biortech.2013.12.031
- [69] Lequette Y, Boels G, Clarisse M, Faille C. Using enzymes to remove biofilms of bacterial isolates sampled in the food-industry. *Biofouling*. 2010;26:421-431. DOI: 10.1080/08927011003699535
- [70] Kumari P, Jandaik S, Batta S. Role of extracellular proteases in biofilm degradation produced from *Escherichia*

coli and *Pseudomonas mendocina*. International Journal of Current Microbiology and Applied Sciences. 2018;**7**:1786-1795. DOI: 10.20546/ijcmas.2018.706.212

[71] Nguyen UT, Burrows LL. DNase I and proteinase K impair *Listeria monocytogenes* biofilm formation and induce dispersal of pre-existing biofilms. International Journal of Food Microbiology. 2014;**187**:26-32. DOI: 10.1016/j.ijfoodmicro.2014.06.025

[72] Johansen C, Falholt P, Gram L. Enzymatic removal and disinfection of bacterial biofilms. Applied and environmental microbiology. 1997;**63**:3724-3728

[73] Wang H, Wang H, Xing T, Wu N, Xu X, Zhou G. Removal of *Salmonella* biofilm formed under meat processing environment by surfactant in combination with bio-enzyme. LWT—Food Science and Technology. 2016;**66**:298-304. DOI: 10.1016/j.lwt.2015.10.049

[74] Tajkarimi MM, Ibrahim SA, Cliver DO. Antimicrobial herb and spice compounds in food. Food Control. 2010;**21**:1199-1218. DOI: 10.1016/j.foodcont.2010.02.003

[75] Nazzaro F, Fratianni F, de Martino L, Coppola R, de Feo V. Effect of essential oils on pathogenic bacteria. Pharmaceuticals (Basel, Switzerland). 2013;**6**:1451-1474. DOI: 10.3390/ph6121451

[76] Swamy MK, Akhtar MS, Sinniah UR. Antimicrobial properties of plant essential oils against human pathogens and their mode of action: An updated review. Evidence-Based Complementary and Alternative Medicine. 2016;**2016**:1-21. DOI: 10.1155/2016/3012462

[77] Leonard CM, Virijevic S, Regnier T, Combrinck S. Bioactivity of selected

essential oils and some components on *Listeria monocytogenes* biofilms. South African Journal of Botany. 2010;**76**:676-680. DOI: 10.1016/j.sajb.2010.07.002

[78] Sandasi M, Leonard CM, Viljoen AM. The effect of five common essential oil components on *Listeria monocytogenes* biofilms. Food Control. 2008;**19**:1070-1075. DOI: 10.1016/j.foodcont.2007.11.006

[79] de Oliveira MMM, Brugnera DF, das Graças Cardoso M, Alves E, Piccoli RH. Disinfectant action of *Cymbopogon* sp. essential oils in different phases of biofilm formation by *Listeria monocytogenes* on stainless steel surface. Food Control. 2010;**21**:549-553. DOI: 10.1016/j.foodcont.2009.08.003

[80] Borugă O, Jianu C, Mișcă C, Goleț I, Gruia AT, Horhat FG. *Thymus vulgaris* essential oil: Chemical composition and antimicrobial activity. Journal of Medicine and Life. 2014;**3**:56-60

[81] Kang J, Liu L, Wu X, Sun Y, Liu Z. Effect of thyme essential oil against *Bacillus cereus* planktonic growth and biofilm formation. Applied Microbiology and Biotechnology. 2018;**102**:10209-10218. DOI: 10.1007/s00253-018-9401-y

[82] Mohsenipour Z, Hassanshahian M. The inhibitory effect of *Thymus vulgaris* extracts on the planktonic form and biofilm structures of six human pathogenic bacteria. Avicenna Journal of Phytomedicine. 2015;**5**:309-318

[83] Szczepanski S, Lipski A. Essential oils show specific inhibiting effects on bacterial biofilm formation. Food Control. 2014;**36**:224-229. DOI: 10.1016/j.foodcont.2013.08.023

[84] Amiri H. Essential oils composition and antioxidant properties of three thymus species. Evidence-based Complementary and Alternative

Medicine: eCAM. 2012;**2012**:1-8. DOI: 10.1155/2012/728065

[85] Pérez-Conesa D, Cao J, Chen L, Mclandsborough L, Weiss J. Inactivation of *Listeria monocytogenes* and *Escherichia coli* O157:H7 biofilms by micelle-encapsulated eugenol and carvacrol. Journal of Food Protection. 2011;**74**:55-62. DOI: 10.4315/0362-028x.jfp-08-403

[86] Knowles JR, Roller S, Murray DB, Naidu AS. Antimicrobial action of carvacrol at different stages of dual-species biofilm development by *Staphylococcus aureus* and *Salmonella enterica* serovar Typhimurium. Applied and Environmental Microbiology. 2005;**71**:797-803. DOI: 10.1128/AEM.71.2.797-803.2005

[87] dos Santos Rodrigues JB, de Carvalho RJ, de Souza NT, de Sousa Oliveira K, Franco OL, Schaffner D, et al. Effects of oregano essential oil and carvacrol on biofilms of *Staphylococcus aureus* from food-contact surfaces. Food Control. 2017;**73**:1237-1246. DOI: 10.1016/j.foodcont.2016.10.043

[88] Miladi H, Zmantar T, Kouidhi B, Chaabouni Y, Mahdouani K, Bakhrouf A, et al. Use of carvacrol, thymol, and eugenol for biofilm eradication and resistance modifying susceptibility of *Salmonella enterica* serovar Typhimurium strains to nalidixic acid. Microbial Pathogenesis. 2017;**104**:56-63. DOI: 10.1016/j.micpath.2017.01.012

[89] Bendre RS, Rajput JD, Bagul SD, Karandikar S. Outlooks on medicinal properties of eugenol and its synthetic derivatives. Natural Products Chemistry & Research. 2016;**4**:1-6. DOI: 10.4172/2329-6836.1000212

[90] Li YH, Tian X. Quorum sensing and bacterial social interactions in biofilms. Sensors (Basel, Switzerland). 2012;**12**:32519-32538. DOI: 10.3390/s120302519

[91] Kim YG, Lee JH, Gwon G, Kim SI, Park JG, Lee J. Essential oils and eugenols inhibit biofilm formation and the virulence of *Escherichia coli* O157:H7. Scientific Reports. 2016;**6**:1-11. DOI: 10.1038/srep36377

[92] Principi N, Silvestri E, Esposito S. Advantages and limitations of bacteriophages for the treatment of bacterial infections. Frontiers in Pharmacology. 2019;**10**:1-9. DOI: 10.3389/fphar.2019.00513

[93] Bai J, Kim YT, Ryu S, Lee JH. Biocontrol and rapid detection of food-borne pathogens using bacteriophages and endolysins. Frontiers in Microbiology. 2016;**7**:1-15. DOI: 10.3389/fmicb.2016.00474

[94] Hagens S, Loessner MJ. Application of bacteriophages for detection and control of foodborne pathogens. Applied Microbiology and Biotechnology. 2007;**76**:513-519. DOI: 10.1007/s00253-007-1031-8

[95] Parasion S, Kwiatek M, Gryko R, Mizak L, Malm A. Bacteriophages as an alternative strategy for fighting biofilm development. Polish Journal of Microbiology. 2014;**63**:137-145

[96] Harper DR, Parracho H, Walker J, Sharp R, Hughes G, Werthén M, et al. Bacteriophages and biofilms. Antibiotics. 2014;**3**:270-284. DOI: 10.3390/antibiotics3030270

[97] Hibma A. Infection and removal of L-forms of *Listeria monocytogenes* with bred bacteriophage. International Journal of Food Microbiology. 1997;**34**:197-207. DOI: 10.1016/s0168-1605(96)01190-7

[98] Soni KA, Nannapaneni R. Removal of *Listeria monocytogenes* biofilms with bacteriophage P100. Journal of Food Protection. 2010;**73**:1519-1524. DOI: 10.4315/0362-028X-73.8.1519

- [99] Arachchi GJG, Cridge AG, Dias-Wanigasekera BM, Cruz CD, McIntyre L, Liu R, et al. Effectiveness of phages in the decontamination of *Listeria monocytogenes* adhered to clean stainless steel, stainless steel coated with fish protein, and as a biofilm. *Journal of Industrial Microbiology & Biotechnology*. 2013;**40**:1105-1116. DOI: 10.1007/s10295-013-1313-3
- [100] Garcia KCOD, Corrêa IMO, Pereira LQ, Silva TM, Mioni MSR, Izidoro ACM, et al. Bacteriophage use to control *Salmonella* biofilm on surfaces present in chicken slaughterhouses. *Poultry Science*. 2017;**96**:3392-3398. DOI: 10.3382/ps/pex124
- [101] Sadekuzzaman M, Yang S, Mizan MFR, Ha SD. Reduction of *Escherichia coli* O157:H7 in biofilms using bacteriophage BPECO 19. *Journal of Food Science*. 2017;**82**:1433-1442. DOI: 10.1111/1750-3841.13729
- [102] Donsì F, Ferrari G. Essential oil nanoemulsions as antimicrobial agents in food. *Journal of Biotechnology*. 2016;**233**:106-120. DOI: 10.1016/j.jbiotec.2016.07.005
- [103] Prakash A, Baskaran R, Paramasivam N, Vadivel V. Essential oil based nanoemulsions to improve the microbial quality of minimally processed fruits and vegetables: A review. *Food Research International*. 2018;**111**:509-523. DOI: 10.1016/j.foodres.2018.05.066
- [104] Moghimi R, Ghaderi L, Rafati H, Aliahmadi A, McClements DJ. Superior antibacterial activity of nanoemulsion of *Thymus daenensis* essential oil against *E. coli*. *Food Chemistry*. 2016;**194**:410-415. DOI: 10.1016/j.foodchem.2015.07.139
- [105] Donsì F, Cuomo A, Marchese E, Ferrari G. Infusion of essential oils for food stabilization: Unraveling the role of nanoemulsion-based delivery systems on mass transfer and antimicrobial activity. *Innovative Food Science & Emerging Technologies*. 2014;**22**:212-220. DOI: 10.1016/j.ifset.2014.01.008
- [106] Shah AA, Khan A, Dwivedi S, Musarrat J, Mint AA. Antibacterial and antibiofilm activity of barium titanate nanoparticles. *Materials Letters*. 2018;**229**:130-133. DOI: 10.1016/j.matlet.2018.06.107
- [107] Prakash A, Vadivel V, Rubini D, Nithyanand P. Antibacterial and antibiofilm activities of linalool nanoemulsions against *Salmonella* Typhimurium. *Food Bioscience*. 2019;**28**:57-65. DOI: 10.1016/j.fbio.2019.01.018
- [108] Gonçalves RC, da Silva DP, Signini R, Naves PLF. Inhibition of bacterial biofilms by carboxymethyl chitosan combined with silver, zinc and copper salts. *International Journal of Biological Macromolecules*. 2017;**105**:385-392. DOI: 10.1016/j.ijbiomac.2017.07.048
- [109] Zanetti M, Carniel TK, Dalcanton F, dos Anjos RS, Gracher Riella H, de Araújo PHH, et al. Use of encapsulated natural compounds as antimicrobial additives in food packaging: A brief review. *Trends in Food Science and Technology*. 2018;**81**:51-60. DOI: 10.1016/j.tifs.2018.09.003
- [110] Subhaswaraj P, Barik S, Macha C, Chiranjeevi PV, Siddhardha B. Anti-quorum sensing and anti-biofilm efficacy of cinnamaldehyde encapsulated chitosan nanoparticles against *Pseudomonas aeruginosa* PAO1. *LWT*. 2018;**97**:752-759. DOI: 10.1016/j.lwt.2018.08.011
- [111] Vijayakumar S, Malaikozhundan B, Saravanakumar K, Durán-Lara EF, Wang MH, Vaseeharan B. Garlic clove extract assisted silver nanoparticle—Antibacterial, antibiofilm,

antihelminthic, anti-inflammatory, anticancer and ecotoxicity assessment. Journal of Photochemistry and Photobiology B: Biology. 2019;**198**:111558. DOI: 10.1016/j.jphotobiol.2019.111558

[112] Comin VM, Lopes LQS, Quatrin PM, de Souza ME, Bonez PC, Pintos FG, et al. Influence of *Melaleuca alternifolia* oil nanoparticles on aspects of *Pseudomonas aeruginosa* biofilm. Microbial Pathogenesis. 2016;**93**:120-125. DOI: 10.1016/j.micpath.2016.01.019

[113] Manju S, Malaikozhundan B, Vijayakumar S, Shanthi S, Jaishabanu A, Ekambaram P, et al. Antibacterial, antibiofilm and cytotoxic effects of *Nigella sativa* essential oil coated gold nanoparticles. Microbial Pathogenesis. 2016;**91**:129-135. DOI: 10.1016/j.micpath.2015.11.021

[114] Wu X, Wang Y, Tao L. Sulfhydryl compounds reduce *Staphylococcus aureus* biofilm formation by inhibiting PIA biosynthesis. FEMS Microbiology Letters. 2011;**316**:44-50. DOI: 10.1111/j.1574-6968.2010.02190.x

[115] Liang Z, Qi Y, Guo S, Hao K, Zhao M, Guo N. Effect of AgWPA nanoparticles on the inhibition of *Staphylococcus aureus* growth in biofilms. Food Control. 2019;**100**:240-246. DOI: 10.1016/j.foodcont.2019.01.030

[116] Naskar A, Khan H, Sarkar R, Kumar S, Halder D, Jana S. Anti-biofilm activity and food packaging application of room temperature solution process based polyethylene glycol capped Ag-ZnO-graphene nanocomposite. Materials Science and Engineering: C. 2018;**C91**:743-753. DOI: 10.1016/j.msec.2018.06.009

[117] Akhil K, Jayakumar J, Gayathri G, Khan SS. Effect of various capping agents on photocatalytic, antibacterial and antibiofilm activities of ZnO nanoparticles. Journal of

Photochemistry and Photobiology B: Biology. 2016;**160**:32-42. DOI: 10.1016/j.jphotobiol.2016.03.015

[118] Pozo NI, Olmos D, Orgaz B, Božanić DK, González-Benito J. Titania nanoparticles prevent development of *Pseudomonas fluorescens* biofilms on polystyrene surfaces. Materials Letters. 2014;**127**:1-3. DOI: 10.1016/j.matlet.2014.04.073

[119] Chorianopoulos NG, Tsoukleris DS, Panagou EZ, Falaras P, Nychas GJE. Use of titanium dioxide (TiO₂) photocatalysts as alternative means for *Listeria monocytogenes* biofilm disinfection in food processing. Food Microbiology. 2011;**28**:164-170. DOI: 10.1016/j.fm.2010.07.025

[120] Goswami SR, Sahareen T, Singh M, Kumar S. Role of biogenic silver nanoparticles in disruption of cell-cell adhesion in *Staphylococcus aureus* and *Escherichia coli* biofilm. Journal of Industrial and Engineering Chemistry. 2015;**26**:73-78. DOI: 10.1016/j.jiec.2014.11.017

[121] Ranmadugala D, Ebrahiminezhad A, Manley-Harris M, Ghasemi Y, Berenjian A. The effect of iron oxide nanoparticles on *Bacillus subtilis* biofilm, growth and viability. Process Biochemistry. 2017;**62**:231-234. DOI: 10.1016/j.procbio.2017.07.003

[122] Govaert M, Smet C, Vergauwen L, Ećimović B, Walsh JL, Baka M, et al. Influence of plasma characteristics on the efficacy of cold atmospheric plasma (CAP) for inactivation of *Listeria monocytogenes* and *Salmonella Typhimurium* biofilms. Innovative Food Science and Emerging Technologies. 2019;**52**:376-386. DOI: 10.1016/j.ifset.2019.01.013

[123] Ziuzina D, Han L, Cullen PJ, Bourke P. Cold plasma inactivation of internalised bacteria and biofilms for *Salmonella enterica* serovar

Typhimurium, *Listeria monocytogenes* and *Escherichia coli*. International Journal of Food Microbiology. 2015;**210**:53-61. DOI: 10.1016/j.ijfoodmicro.2015.05.019

[124] Patange A, Boehm D, Ziuzina D, Cullen PJ, Gilmore B, Bourke P. High voltage atmospheric cold air plasma control of bacterial biofilms on fresh produce. International Journal of Food Microbiology. 2019;**293**:137-145. DOI: 10.1016/j.ijfoodmicro.2019.01.005

[125] Czapka T, Maliszewska I, Olesiak-Bańska J. Influence of atmospheric pressure non-thermal plasma on inactivation of biofilm cells. Plasma Chemistry and Plasma Processing. 2018;**38**:1181-1197. DOI: 10.1007/s11090-018-9925-z

[126] Koban I, Geisel MH, Holtfreter B, Jablonowski L, Hübner N-O, Matthes R, et al. Synergistic effects of nonthermal plasma and disinfecting agents against dental biofilms *in vitro*. ISRN Dentistry. 2013;**2013**:1-10. DOI: 10.1155/2013/573262

[127] Gupta TT, Karki SB, Matson JS, Gehling DJ, Ayan H. Sterilization of biofilm on a titanium surface using a combination of nonthermal plasma and chlorhexidine digluconate. BioMed Research International. 2017;**2017**:6085741. DOI: 10.1155/2017/6085741

[128] Múgica-Vidal R, Sainz-García E, Álvarez-Ordóñez A, Prieto M, González-Raurich M, López M, et al. Production of antibacterial coatings through atmospheric pressure plasma: A promising alternative for combatting biofilms in the food industry. Food and Bioprocess Technology. 2019;**12**:1251-1263. DOI: 10.1007/s11947-019-02293-z

[129] Cappitelli F, Polo A, Villa F. Biofilm formation in food processing environments is still poorly understood and controlled. Food Engineering

Reviews. 2014;**6**:29-42. DOI: 10.1007/s12393-014-9077-8

[130] Oulahal-Lagsir N, Martial-Gros A, Bonneau M, Blum LJ. *Escherichia coli*-milk biofilm removal from stainless steel surfaces: Synergism between ultrasonic waves and enzymes. Biofouling. 2003;**19**(3):159-168. DOI: 10.1080/0892701031000064676

[131] Oulahal N, Martial-Gros A, Bonneau M, Blum LJ. Combined effect of chelating agents and ultrasound on biofilm removal from stainless steel surfaces. Application to “*Escherichia coli* milk” and “*Staphylococcus aureus* milk” biofilms. Biofilms. 2004;**1**:65-73. DOI: 10.1017/s1479050504001140

[132] Hussain MS, Kwon M, Park E, Seheli K, Huque R, Oh DH. Disinfection of *Bacillus cereus* biofilms on leafy green vegetables with slightly acidic electrolyzed water, ultrasound and mild heat. Lwt. 2019;**116**:108582. DOI: 10.1016/j.lwt.2019.108582

[133] Bang HJ, Park SY, Kim SE, Md Furkanur Rahaman M, Ha SD. Synergistic effects of combined ultrasound and peroxyacetic acid treatments against *Cronobacter sakazakii* biofilms on fresh cucumber. LWT—Food Science and Technology. 2017;**84**:91-98. DOI: 10.1016/j.lwt.2017.05.037

[134] Fink R, Oder M, Stražar E, Filip S. Efficacy of cleaning methods for the removal of *Bacillus cereus* biofilm from polyurethane conveyor belts in bakeries. Food Control. 2017;**80**:267-272. DOI: 10.1016/j.foodcont.2017.05.009

[135] Pedrós-Garrido S, Condón-Abanto S, Clemente I, Beltrán JA, Lyng JG, Bolton D, et al. Efficacy of ultraviolet light (UV-C) and pulsed light (PL) for the microbiological decontamination of raw salmon (*Salmo salar*) and food contact surface materials. Innovative Food Science and

Emerging Technologies. 2018;**50**:124-131. DOI: 10.1016/j.ifset.2018.10.001

[136] Turtoi M, Borda D. Decontamination of egg shells using ultraviolet light treatment. World's Poultry Science Journal. 2014;**70**(2):265-278. DOI: 10.1017/S0043933914000282

[137] Mahendran R, Ramanan KR, Barba FJ, Lorenzo JM, López-Fernández O, Munekata PES, et al. Recent advances in the application of pulsed light processing for improving food safety and increasing shelf life. Trends in Food Science and Technology. 2019;**88**:67-79. DOI: 10.1016/j.tifs.2019.03.010

[138] Garvey M, Rowan NJ. Pulsed UV as a potential surface sanitizer in food production processes to ensure consumer safety. Current Opinion in Food Science. 2019;**26**:65-70. DOI: 10.1016/j.cofs.2019.03.003

[139] Rajkovic A, Tomasevic I, De Meulenaer B, Devlieghere F. The effect of pulsed UV light on *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella* Typhimurium, *Staphylococcus aureus* and staphylococcal enterotoxin A on sliced fermented salami and its chemical quality. Food Control. 2017;**73**:829-837. DOI: 10.1016/j.foodcont.2016.09.029

[140] Cossu A, Ercan D, Wang Q, Peer WA, Nitin N, Tikekar RV. Antimicrobial effect of synergistic interaction between UV-A light and gallic acid against *Escherichia coli* O157:H7 in fresh produce wash water and biofilm. Innovative Food Science and Emerging Technologies. 2016;**37**:44-52. DOI: 10.1016/j.ifset.2016.07.020

[141] Gora SL, Rauch KD, Ontiveros CC, Stoddart AK, Gagnon GA. Inactivation of biofilm-bound *Pseudomonas aeruginosa* bacteria using UVC light emitting diodes (UVC LEDs). Water Research. 2019;**151**:193-202. DOI: 10.1016/j.watres.2018.12.021

[142] Garner AL. Pulsed electric field inactivation of microorganisms: From fundamental biophysics to synergistic treatments. Applied Microbiology and Biotechnology. 2019;**103**:7917-7929. DOI: 10.1007/s00253-019-10067-y

[143] Halpin RM, Duffy L, Cregenzán-Alberti O, Lyng JG, Noci F. The effect of non-thermal processing technologies on microbial inactivation: An investigation into sub-lethal injury of *Escherichia coli* and *Pseudomonas fluorescens*. Food Control. 2014;**41**:106-115. DOI: 10.1016/j.foodcont.2014.01.011

[144] Monfort S, Gayán E, Saldaña G, Puértolas E, Condón S, Raso J, et al. Inactivation of *Salmonella* Typhimurium and *Staphylococcus aureus* by pulsed electric fields in liquid whole egg. Innovative Food Science and Emerging Technologies. 2010;**11**:306-313. DOI: 10.1016/j.ifset.2009.11.007

[145] Bleoancă I, Saje K, Mihalcea L, Oniciuc EA, Smole-Mozina S, Nicolau AI, et al. Contribution of high pressure and thyme extract to control *Listeria monocytogenes* in fresh cheese—A hurdle approach. Innovative Food Science and Emerging Technologies. 2016;**38**:7-14. DOI: 10.1016/j.ifset.2016.09.002

[146] Buckow R, Weiss U, Knorr D. Inactivation kinetics of apple polyphenol oxidase in different pressure-temperature domains. Innovative Food Science & Emerging Technologies. 2009;**10**:441-448. DOI: 10.1016/j.ifset.2009.05.005

[147] Zetzmann M, Bucur FI, Crauwels P, Borda D, Nicolau AI, Grigore-Gurgu L, et al. Characterization of the biofilm phenotype of a *Listeria monocytogenes* mutant deficient in *agr* peptide sensing. MicrobiologyOpen. 2019;**8**:1-9. DOI: 10.1002/mbo3.826

[148] Gross M, Cramton SE, Gotz F, Peschel A. Key role of teichoic acid

net charge in *Staphylococcus aureus* colonization of artificial surfaces. *Infection and Immunity*. 2001;**69**:3423-3426. DOI: 10.1128/IAI.69.5.3423-3426.2001

[149] Kot B, Sytykiewicz H, Sprawka I. Expression of the biofilm-associated genes in methicillin-resistant *Staphylococcus aureus* in biofilm and planktonic conditions. *International Journal of Molecular Science*. 2018;**19**(11):3487. DOI: 10.3390/ijms19113487

[150] Hecker M, Mäder U, Völker U. From the genome sequence via the proteome to cell physiology—Pathoproteomics and pathophysiology of *Staphylococcus aureus*. *International Journal of Medical Microbiology*. 2018;**308**:545-557. DOI: 10.1016/j.ijmm.2018.01.002

[151] Yang YH, Jiang YL, Zhang J, Wang L, Bai XH, Zhang SJ, et al. Structural insights into SraP-mediated *Staphylococcus aureus* adhesion to host cells. *PLoS Pathogens*. 2014;**10**:e1004169. DOI: 10.1371/journal.ppat.1004169

[152] Chastanet A, Losick R. Just-in-time control of spo0A synthesis in *Bacillus subtilis* by multiple regulatory mechanisms. *Journal of Bacteriology*. 2011;**193**:6366-6374. DOI: 10.1128/JB.06057-11

[153] Mirouze N, Prepiak P, Dubnau D. Fluctuations in spo0A transcription control rare developmental transitions in *Bacillus subtilis*. *PLoS Genetics*. 2011;**7**:e1002048. DOI: 10.1371/journal.pgen.1002048

[154] Jers C, Kobir A, Sondergaard EO, Jensen PR, Mijakovic I. *Bacillus subtilis* two-component system sensory kinase DegS is regulated by serine phosphorylation in its input domain. *PLoS One*. 2011;**6**:E14653-E14653. DOI: 10.1371/journal.pone.0014653

[155] Murray EJ, Kiley TB, Stanley-Wall NR. A pivotal role for the response regulator DegU in controlling multicellular behaviour. *Microbiology*. 2009;**155**:1-8. DOI: 10.1099/mic.0.023903-0

[156] Spöring I, Felgner S, Preuß M, Eckweiler D, Rohde M, Häussler S, et al. Regulation of flagellum biosynthesis in response to cell envelope stress in *Salmonella enterica* serovar Typhimurium. *MBio*. 2018;**9**(3):e00736-e00717. DOI: 10.1128/mBio.00736-17

[157] Alikhan NF, Zhou Z, Sergeant MJ, Achtman M. A genomic overview of the population structure of *Salmonella*. *PLOS Genetics*. 2018;**14**:e1007261. DOI: 10.1371/journal.pgen.1007261

[158] Barbu EM, Ganesh VK, Gurusiddappa S, Mackenzie RC, Foster TJ, Sudhof TC, et al. β -Neurexin is a ligand for the *Staphylococcus aureus* MSCRAMM SdrC. *PLoS Pathogens*. 2010;**6**:e1000726. DOI: 10.1371/journal.ppat.1000726

[159] Zhang X-S, García-Contreras R, Wood TK. YcfR (BhsA) influences *Escherichia coli* biofilm formation through stress response and surface hydrophobicity. *Journal of Bacteriology*. 2007;**189**:3051-3062. DOI: 10.1128/JB.01832-06

[160] Weber MM, French CL, Barnes MB, Siegele DA, McLean RJ. A previously uncharacterized gene, yjfo (bsmA), influences *Escherichia coli* biofilm formation and stress response. *Microbiology*. 2010;**156**:139-147. DOI: 10.1099/mic.0.031468-0

[161] Grantcharova N, Peters V, Monteiro C, Zakikhany K, Römling U. Bistable expression of CsgD in biofilm development of *Salmonella enterica* Seroovar Typhimurium. *Journal of Bacteriology*. 2009;**192**:456-466. DOI: 10.1128/JB.01826-08

- [162] Brown PK, Dozois CM, Nickerson CA, Zuppardo A, Terlonge J, Curtiss R 3rd. MlrA, a novel regulator of curli (AgF) and extracellular matrix synthesis by *Escherichia coli* and *Salmonella enterica* serovar Typhimurium. *Molecular Microbiology*. 2001;**41**:349-363. DOI: 10.1046/j.1365-2958.2001.02529.x
- [163] Kearns DB, Chu F, Branda SS, Kolter R, Losick R. A master regulator for biofilm formation by *Bacillus subtilis*. *Molecular Microbiology*. 2005;**55**:739-749. DOI: 10.1111/j.1365-2958.2004.04440.x
- [164] Kodgire P, Dixit M, Rao KKJ. ScoC and SinR negatively regulate epr by corepression in *Bacillus subtilis*. *Journal of Bacteriology*. 2006;**188**:6425-6428. DOI: 10.1128/JB.00427-06
- [165] López D, Vlamakis H, Losick R, Kolter R. Cannibalism enhances biofilm development in *Bacillus subtilis*. *Molecular Microbiology*. 2009;**74**:609-618. DOI: 10.1111/j.1365-2958.2009.06882.x
- [166] Vlamakis H, Chai Y, Beauregard P, Losick R, Kolter R. Sticking together: Building a biofilm the *Bacillus subtilis* way. *Nature Reviews. Microbiology*. 2013;**11**(3):157-168. DOI: 10.1038/nrmicro2960
- [167] Lazarevic V, Soldo B, Médico N, Pooley H, Bron S, Karamata D. *Bacillus subtilis* α -phosphoglucomutase is required for normal cell morphology and biofilm formation. *Applied and Environmental Microbiology*. 2005;**71**:39-45. DOI: 10.1128/AEM.71.1.39-45.2005
- [168] Hobley L, Li B, Wood JL, Kim SH, Naidoo J, Ferreira AS, et al. Spermidine promotes *Bacillus subtilis* biofilm formation by activating expression of the matrix regulator slrR. *Journal of Biological Chemistry*. 2017;**292**:12041-12053. DOI: 10.1074/jbc.M117.789644
- [169] Branda SS, Chu F, Kearns DB, Losick R, Kolter R. A major protein component of the *Bacillus subtilis* biofilm matrix. *Molecular Microbiology*. 2006;**59**:1229-1238. DOI: 10.1111/j.1365-2958.2005.05020.x
- [170] Kobayashi K, Iwano M. BslA(YuaB) forms a hydrophobic layer on the surface of *Bacillus subtilis* biofilms. *Molecular Microbiology*. 2012;**85**:51-66. DOI: 10.1111/j.1365-2958.2012.08094.x
- [171] Hobley L, Ostrowski A, Rao FV, Bromley KM, Porter M, Prescott AR, et al. BslA is a self-assembling bacterial hydrophobin that coats the *Bacillus subtilis* biofilm. *Proceedings of the National Academy of Sciences of the United States of America*. 2013;**110**:13600-13605. DOI: 10.1073/pnas.1306390110
- [172] Zhang J, Poh CL. Regulating exopolysaccharide gene wcaF allows control of *Escherichia coli* biofilm formation. *Scientific Reports*. 2018;**8**:13127. DOI: 10.1038/s41598-018-31161-7
- [173] Pando JM, Karlinsey JE, Lara JC, Libby SJ, Fang FC. The Rcs-regulated colanic acid capsule maintains membrane potential in *Salmonella enterica* serovar Typhimurium. *American Society of Microbiology*. 2007;**8**(3):e00808-e00817. DOI: 10.1128/mBio.00808-17
- [174] Ma Q, Yang Z, Pu M, Peti W, Wood TK. Engineering a novel c-di-GMP-binding protein for biofilm dispersal. *Environ. Microbiology*. 2011;**13**:631-642. DOI: 10.1111/j.1462-2920.2010.02368.x
- [175] Cucarella C, Solano C, Valle J, Amorena B, Lasa I, Penades JR. Bap, a *Staphylococcus aureus* surface protein involved in biofilm formation. *Journal of Bacteriology*. 2001;**183**:2888-2896. DOI: 10.1128/JB.183.9.2888-2896.2001

- [176] Latasa C, Roux A, Toledo-Arana A, Ghigo J-M, Gamazo C, Penadés JR, et al. BapA, a large secreted protein required for biofilm formation and host colonization of *Salmonella enterica* serovar Enteritidis. *Molecular Microbiology*. 2005;58:1322-1339. DOI: 10.1111/j.1365-2958.2005.04907.x
- [177] Silva-Hidalgo G, López-Valenzuela M, Cárcamo-Aréchiga N, Cota-Guajardo S, López-Salazar M, Montiel-Vázquez E. Identification of bapA in strains of *Salmonella enterica* subsp. *enterica* isolated from wild animals kept in captivity in Sinaloa, Mexico. *Veterinary Medicine International*. 2016;3478746. DOI: 10.1155/2016/3478746
- [178] Hartford OM, Wann ER, Höök M, Foster TJ. Identification of residues in the *Staphylococcus aureus* fibrinogen-binding MSCRAMM clumping factor a (ClfA) that are important for ligand binding. *Journal of Biological Chemistry*. 2001;276:2466-2473. DOI: 10.1074/jbc.M007979200
- [179] Dunman PM, Murphy E, Haney S, Palacios D, Tucker-Kellogg G, Wu S, et al. Transcription profiling-based identification of *Staphylococcus aureus* genes regulated by the agr and/or sarA loci. *Journal of Bacteriology*. 2001;183:7341-7353. DOI: 10.1128/JB.183.24.7341-7353.2001
- [180] Chen J, Xia Y, Cheng C, Fang C, Shan Y, Jin G, et al. Genome sequence of the nonpathogenic *Listeria monocytogenes* Serovar 4a strain M7. *Journal of Bacteriology*. 2011;193:5019-5020. DOI: 10.1128/JB.05501-11
- [181] Miller MB, Bassler BL. Quorum sensing in bacteria. *Annual Review of Microbiology*. 2001;55:165-199. DOI: 10.1146/annurev.micro.55.1.165
- [182] Gomez MI, Seaghdha MO, Prince AS. *Staphylococcus aureus* protein a activates TACE through EGFR-dependent signaling. *The EMBO Journal*. 2007;26:701-709. DOI: 10.1038/sj.emboj.7601554
- [183] Weinmaier T, Riesing M, Rattei T, Bille J, Arguedas-Villa C, Stephan R, et al. The complete genome sequence of *Listeria monocytogenes* LL195—A serotype 4b strain from the 1983 to 1987 listeriosis epidemic in Switzerland. *Genome Announcements*. 2013;1:e00152-e00112. DOI: 10.1128/genomeA.00152-12
- [184] Park C, Shin NY, Byun JH, Shin HH, Kwon EY, Choi SM, et al. Down regulation of RNAIII in vancomycin-intermediate *Staphylococcus aureus* strains regardless of the presence of agr mutation. *Journal of Medical Microbiology*. 2012;61:345-352. DOI: 10.1099/jmm.0.035204-0
- [185] Lou Z, Chen J, Yu F, Wang H, Kou X, Ma C, et al. The antioxidant, antibacterial, antibiofilm activity of essential oil from *Citrus medica* L. var. *sarcodactylis* and its nanoemulsion. *LWT—Food Science and Technology*. 2017;80:371-377. DOI: 10.1016/j.lwt.2017.02.037
- [186] da Silva Gündel S, de Souza ME, Quatrin PM, Klein B, Wagner R, Gündel A, et al. Nanoemulsions containing *Cymbopogon flexuosus* essential oil: Development, characterization, stability study and evaluation of antimicrobial and antibiofilm activities. *Microbial Pathogenesis*. 2018;118:268-276. DOI: 10.1016/j.micpath.2018.03.043
- [187] Letsididi K, Lou Z, Letsididi R, Mohammed K, Maguy B. Antimicrobial and antibiofilm effects of trans-cinnamic acid nanoemulsion and its potential application on lettuce. *LWT—Food Science and Technology*. 2018;94:25-32. DOI: 10.1016/j.lwt.2018.04.018