Chapter

Biofilms Formed by Pathogens in Food and Food Processing Environments

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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 *Staphylo-coccus* 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% O_2 and 10% CO_2) and in aerobic conditions (20% O_2) [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 (*fli*D, *fli*Q, *fla*A, and *mot*A), a required property for initial surface

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

Gene/KEGG/protein encoded	Gene function	Role	Bacterium	Ref.
Microcolonies development				
*dlt*A/LMOf2365_099/D-alanine-D-alanyl carrier protein ligase	Catalyzes the first step in the D-alanylation of lipoteichoic acid (LTA)	Cell wall biogenesis	*L. monocytogenes S. aureus*	[24]
*dlt*C/LMOf2365_099/D-alanyl carrier protein	Carrier protein involved in the D-alanylation of LTA			
*dlt*B/lmo0973/DltB	Involved in the transport of activated D-alanine through the membrane		*S. aureus, B. subtilis*	
sdrC/NWMN_0523/Serine-aspartate repeat-containing protein C *sdrH*/SAUSA300_1985 Serine-aspartate repeat family protein	Cell adhesion	Mediates interactions with components of the extracellular matrix to promote bacterial adhesion	*S. aureus*	[158]
*bhs*A/STY1254/Multiple stress resistance protein	Stress response, response to copper ion	Regulation of biofilm formation. May repress cell–cell interaction and cell surface interaction	*E. coli*	[159]
*bsm*A/*yjf*O/Lipoprotein	Stress response to hydrogen peroxide and to DNA damage	Single-species biofilm formation; enhanced flagellar motility	*E. coli*, *S. enterica*	[160]
*csg*D/b1040/CsgBAC operon transcriptional regulatory protein	DNA-binding transcription activator activity	The master regulator for adhesive curli fimbriae expression		[161]
*mlr*A/b2127/HTH-type transcriptional regulator	DNA-binding transcription factor activity	Activates transcription of *csg*D		[162]
*sin*R/BSU24610/HTH-type transcriptional regulator	Negatively regulates transcription of the *eps* operon	DNA-binding protein master regulator of biofilm formation	*B. subtilis*, *B. cereus*	[163, 164]
*eps*G (*yve*Q)/BSU34310/Transmembrane protein	Production of exopolysaccharide	Biofilm maintenance		[165]
*eps*H (*yve*R)/BSU34300/Putative glycosyl-transferase				[166]
*ymd*B/BSU16970/2′,3′-cyclic-nucleotide 2′-phospho-diesterase	Regulatory role. Induces genes involved in biofilm formation	Directing the early stages of colony development		
*pgc*A/Phosphoglucomutase	Catalyzes the interconversion between glucose-6-phosphate and alpha-glucose-1-phosphate	Exopolysaccharide synthesis		[167]

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

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

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 *prf*A, 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 (*pro*P, *opu*D, *aap*A, and *dlt*A) were upregulated after 8 hours from the biofilm initiation [29]. For *E. coli*, the genes involved in the cell adhesion, like *fim*A, *yad*K, *yad*N, *yad*M, and *yad*C-encoding fimbriae-like proteins-are coexpressed with the integral cell membrane genes, with outer membrane proteins (*htr*E), with transcriptional regulators (*mng*R and *nha*R), or other genes, but this network appears to be strain specific [30, 31].

In the case of *S. enterica*, differential expression analysis revealed that *ycf*R 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 *csg*BAC operon, which encodes the structural subunits for curli, indirectly activates the transcription of the second mechanism, *adr*A, 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 Δ*dlt*ABC *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 *tap*A and *bsl*A from *B. subtilis* genome are absent in the matrix of *B. cereus* because these genes have no paralog in *B. cereus* genome. Instead the *tas*A 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 *wca*L gene (*S. enterica*) or *wca*F (*E. coli*). It has been also shown that *rpo*S 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, *lux*S and *lux*R, homolog to *Sdi*A 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 a*ctA*, 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]. EOs 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 ~9 log10 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 *Citrus medica* L. var. *sarcodactylis*	73	*S. aureus*	Inhibit the ability of bacteria to attach to surfaces	[185]
EO of *Cymbopogon flexuosus* (lemongrass)	78.46 ± 0.51	*P. aeruginosa* (PA01) and *S. aureus* (ATCC 29213)	Reduce the adhesion of pathogenic bacteria to surfaces	[186]
Trans-CA	>100 <100	*P. aeruginosa* (CMCC 10104), *S.* typhimurium and *S. aureus*	Membrane disruption by destabilization of lipids	[187]
Linalool	10.9 ± 0.1	*S.* typhimurium (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 TiO_2/polystyrene nanocomposite film [118]. It has been shown that nanostructured TiO_2 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/cm^2), but the highest reduction was obtained for a DBD electrode with He and no O_2 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/cm^2) 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/cm^2 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* 0157:H7, *L. monocytogenes*, and *S. aureus* on the surface of dry fermented salami inoculated with 6.3 log CFU/g at 3 J/cm^2 (1 pulse) or 15 J/cm^2 (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 Δ*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.

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