ISSN 0026-2617, Microbiology, 2014, Vol. 83, No. 6, pp. 713–722. © Pleiades Publishing, Ltd., 2014.

REVIEWS

Composition and Functions of the Extracellular Polymer Matrix of Bacterial Biofilms

M. V. Zhurina[a], A. V. Gannesen[a], E. L. Zdorovenko[b], and V. K. Plakunov[a, 1]

[a] *Winogradsky Institute of Microbiology, Russian Academy of Sciences, pr. 60-letiya Oktyabrya 7, k. 2, Moscow, 117312 Russia*

[b] *Zelinsky Institute of Organic Chemistry, Russian Academy of Sciences, Moscow, Russia*

Received December 24, 2013

Abstract—The data on the composition and structure of the components comprising the extracellular polymer matrix of bacterial biofilms, the role of these components, and their functions in the biofilm are reviewed. The main biochemical mechanisms regulating the biosynthesis of biofilm matrix are discussed.

Keywords: biofilms, matrix components, polysaccharides, proteins, DNA, regulatory mechanisms

DOI: 10.1134/S002626171406023X

A biofilm form of microbial existence implies the presence of an extracellular polymer matrix (EPM) in which microbial microcolonies are embedded [1–3].

COMPOSITION AND STRUCTURE OF THE EXTRACELLULAR POLYMER MATRIX

While the EPM composition and structure in different microorganisms may differ significantly (both qualitatively and quantitatively), polysaccharides are always their major components. The goal of the present review is to analyze the recent publications concerning the composition, structure, and functions of the EPM components, as well as the main mechanisms for the regulation of their synthesis.

Polysaccharides of Gram-Negative Bacteria

Discussion of lipopolysaccharides, both cell-bound and those present in the biofilm matrix, is not the goal of this review, although these compounds play an important role in pathogenesis and carry out antigenic functions [4, 5]. We will concentrate on the extracellular polysaccharides of the biofilm EPM.

Unfortunately, the presently available information concerning the mechanisms of matrix formation and functioning is insufficient. Matrix composition has been most extensively studied for the biofilms produced by gram-negative bacteria (especially pseudomonads) and by some gram-positive bacteria.

P. aeruginosa, the causative agent for chronic lung infections in persons with hereditary malfunction of the endocrine system (mucoviscidosis) and the organism that forms biofilms on contact lenses, catheters, and other implanted medical devices, is the best studied object [6]. The EPM of *P. aeruginosa* biofilms is a complex structure comprising exopolysaccharides, proteins, and nucleic acids (primarily DNA) [7].

The matrix of *P. aeruginosa* biofilms contains three major exopolysaccharides—alginate, Psl, and Pel (the latter contains glucose as the major component). The role of minor components of polysaccharide nature, such as cyclic β-glucans and levan, will be described below and in the EMP Functions section.

Alginate. This linear polyanionic acidic polysaccharide is a high-molecular mass product, in which residues of L-glucuronic and D-mannuronic acids are connected with β-1,4 bonds. It is usually acetylated in positions 2 or 3 of the mannuronic acid residue. The 1,4 bond makes the polymer more rigid than dextrans, where the 1,2 bond is present [8]. While alginate is usually accumulated in the biofilms of clinical mucoid *P. aeruginosa* isolates, it is also produced by nonpathogenic variants of pseudomonads. Since expression of the genes of alginate biosynthesis was not observed in the course of biofilm formation by *P. aeruginosa* strains PAO1 and PA14, it is not a necessary biofilm component under in vitro conditions [9]. Detailed research by a variety of physicochemical techniques revealed that alginate increased EPM hydratation, decreased its flexibility, and screened the surface structures and adhesins, thus regulating the interaction between the cells and the substrate surface [10]. The role of alginate was studied in more detail in subsequent works. Mucoid features associated with enhanced alginate production were found to develop in nonmucoid *P. aeruginosa* strains (PAO1, PA14, PAKS-1, and Ps388) after mutation of one of the genes of the *muc* operon (*mucA*, *mucB*, *mucC*, or *mucD*), although only under iron limitation in the medium (below 5 μM), while high alginate production by clinical strains isolated from mucoviscidosis

[1] Corresponding author; e-mail: plakunov@inmi.host.ru

patients did not depend on iron concentration in the medium [11]. Moreover, it was recently shown that in *P. alkylphenolia* the cluster of *epm* (extracellular polymer matrix) genes (which are homologous to the genes of alginate biosynthesis and have been revealed in some *Pseudomonas*, *Alcanivorax*, and *Marinobacter* species) encodes the synthesis of substituted polymannuronic acid, an analogue of the mannuronic acid of alginate. Expression of these genes is required for formation of specialized biofilms by this organism [12].

Levan, or β-polyfructan, another capsular polysaccharide of pseudomonads, is considered as a storage, rather than structural, biofilm polysaccharide, although its possible role in resistance of *P. brassicacearum* biofilms to osmotic and oxidative stress caused by Cd^{2+} ions has been hypothesized [13].

Psl polysaccharide. Mannose was the main component of this polysaccharide revealed by early studies. Later research showed, however, that Psl consisted of a repeated pentasaccharide, including three D-mannose residues and one residue of both D-glucose and L-rhamnose bound by 1,3 bonds. At least two forms of this polysaccharide exist: a high-molecular mass component associated with biofilms and a soluble component of relatively low molecular mass, which is present in the culture liquid [8]. The role of the Psl exopolysaccharide, which is located at the cell surface as spiral structures and ensures intercellular interactions within a biofilm, has been studied in some detail. Psl is involved in formation of cavities in three-dimensional microcolonies during the maturation of biofilms. These cavities harbor motile cells responsible for the propagation of bacteria in the course of dissociation of the biofilm. Chemical removal of Psl from the surface of bacterial cells resulted in decomposition of the matrix [14]. The *psl* locus contains 15 co-transcribed genes homologous to the known genes of polysaccharide biosynthesis, although only 11 of them are required for biofilm formation [15]. Induced expression of the *psl* locus occurs in response to high levels of a global regulator cyclic diguanosine monophosphate (c-di-GMP). The alternative sigma factor RpoS was shown to induce transcription of the *psl* locus during the stationary growth phase, while the RNA-binding protein RsmA is known to suppress translation of the messenger RNA from the *psl* locus by binding to its untranslated 5' end [16]. Detailed investigation of the biosynthesis of polysaccharides (alginate, Psl, and Pel) are presented in a recent work [17].

Pel polysaccharide. Similar to cellulose, this polysaccharide consists of glucose residues and is stained with Congo red. Its exact structure, however, has not been established. Pel forms the structural framework of the matrix and provides for the interactions of microbial cells, especially at the early stages of biofilm formation by the *P. aeruginosa* PA14 mutant incapable of Psl synthesis [18]. Phenotypically, enhanced Pel synthesis results in formation of wrinkled colonies [19]. Pel was shown to provide for microbial attachment to glass surface in experiments in vitro, although the effect was less pronounced than in the case of Psl. However, synergism of Pel and Psl was observed when both of them were present [20]. The *P. aeruginosa* PA14 mutant unable to synthesize Pel exhibited decreased binding of the core oligosaccharide of the outer membrane lipopolysaccharide to the cell. An operon consisting of seven genes is required for involvement of Pel participates in biofilm formation: these genes affect the synthesis, transport, or processing of polysaccharides. Synthesis of extracellular polysaccharides usually occurs in five stages. A precursor substrate (a nucleotide-activated sugar) is synthesized in the cytoplasm. It is subsequently polymerized with formation of a growing polysaccharide chain. The polysaccharide is then transported through the cytoplasmic membrane into the periplasm, where it may undergo enzymatic modification. It is finally exported though the outer membrane into the environment. Deacetylation probably occurs in the course of Pel synthesis. The role of this modification for Pel functioning in biofilms remains unknown [18].

Cellulose. The role of cellulose and colanic acid for biofilm formation was studied in detail in enteric bacteria, for which they are the major polysaccharide components [21]. Some information, however, was recently obtained concerning the role of cellulose as an EPM component in pseudomonads. The role of cellulose in biofilm formation was especially well studied for *P. fluorescens* and *P. putida*. Both organisms contain the *wss* operon or its homologues, the products of which are involved in cellulose biosynthesis. Biofilm formation by *P. fluorescens*, especially at the air–liquid (A–L) interface, depends on cellulose [22]. *P. putida* has no *psl* locus, and bacterial cellulose and polysaccharide A play an important role in maintaining biofilm stability [23].

It was recently found that c-di-GMP stimulated cellulose synthesis by bacteria. The cellulose molecule is synthesized in the cytoplasm and transported through the cytoplasmic membrane by the BcsA and BcsB subunits of cellulose synthetase. It was shown that c-di-GMP releases the enzyme from its self-inhibited state by disrupting the salt bridge blocking its active center. A similar result may be achieved by mutagenesis. Moreover, c-di-GMP carries out allosteric activation of the BcsA–BcsB complex by modulation of the active center of the enzyme [24].

Rhamnolipids. These compounds form a class of amphiphilic surface-active glycolipids containing rhamnose and fatty acids. They have been most completely characterized for bacteria of the genera *Pseudomonas* and *Burkholderia*. Over 60 compounds belonging to rhamnolipids have been identified.

P. aeruginosa produces a mixture of four major components: two dimeric rhamnolipids, α-L-rhamnopyranosyl-α-L-rhamnopyranosyl-β-hydroxydecanoyl-β-hydroxydecanoate (Rha-Rha-C10-C10) and α-L-

rhamnopynanosyl-α-L-rhamnopyranosyl-β-hydroxydecanoate (Rha-Rha-C10), and two related monomeric derivatives, Rha-C10-C10 and Rha-C10 [25]. The terminal stages of rhamnolipid synthesis by this microorganism are controlled by three genes in two independent operons: the *rhlAB* genes in one of them and the *rhlC* gene in the other. Various *Burkholderia* species were found to possess closely related genes, which were, however, grouped in one operon [26]. Rhamnolipids have certain advantages compared to other microbial surfactants: they are easily biodegradable, have low toxicity, and may be produced from renewable materials. In rhamnolipid-producing organisms, they facilitate sorption and biodegradation of poorly soluble substrates, act as immunomodulators and pathogenicity factors, and also possess antimicrobial and antifungal activity. They affect the capacity of producers for movement along phase boundaries and for biofilm formation [27].

Polysaccharides and Related Compounds in Gram-Positive Bacteria

Among gram-positive bacteria, biofilm formation has been extensively studied in *Bacillus* spp. (with *B. subtilis* as a model organism), *Staphylococcus* spp., and *Streptococcus* spp. The latter genus is of special importance due to its role in formation of multispecies biofilms (dental plaques), in development of nosocomial infections, and in overgrowth upon the surfaces of implanted medical equipment [28].

Levan, which has been mentioned among the *Pseudomonas* polysaccharides, is the best studied exopolysaccharide produced by *B. subtilis*. Two types of *B. subtilis* levans are known. Type I consists of D-fructose residues bound with β-2,6 bonds. Type II levan is a fructose polymer with glucose bound to its terminal residues by α-glucoside bonds. Some *B. subtilis* strains were found to produce exopolysaccharides of different structure, in which glucose, galactose, fructose, glucuronic acid, and *O*-acetyl groups are present in approximate molar ratios of 2 : 2 : 1 : 1 : 1.5 [29].

Products of the *epsA-epsO* operon are responsible for synthesis of the major exopolysaccharide components required for biofilm formation. Mutation in any of these genes results in impaired biofilm formation. Mutations in the *pgcA* gene (previously designated as *yhxB*), which encodes α-phosphoglucomutase, and in the *gtaB* gene encoding UTP-glucose-1-phosphate-uridilyl transferase (required for nucleotide sugar phosphates involved in exopolysaccharide biosynthesis) have a similar effect [30].

Apart from exopolysaccharides, *B. subtilis* EPM contains an anionic polymer poly-D-glutamate. While the ratio of these polymers varies in different strains, both are required for biofilm formation.

Most *S. aureus* strains form PNAG, an *N*-acetylglucosamine polymer, which participates in biofilm formation, acting, among other things, as an adhesin. PNAG is synthesized by four proteins: IcaA, IcaD, IcaB, and IcaC, encoded by the *ica* operon, which is downregulated by the IcaR repressor, encoded by the divergently expressed gene *icaR* [31]. However, deficiency in the *ica* operon responsible for the synthesis of this polysaccharide may be compensated for by formation of various proteinaceous adhesins (biofilm associated proteins, Bap) [32].

Unlike *S. aureus*, *B. subtilis* has only one protein with adhesin functions (TasA) in its biofilm matrix. Mutants with impaired synthesis of this protein are unable to form biofilms, in spite of their retained capacity for exopolysaccharide synthesis. Existence of TasA as extracellular filaments with the properties of an amyloid protein playing an important role in the biofilm matrix was recently demonstrated [33].

In the case of biofilms (dental plaques) formed by *S. mutans*, polysaccharides forming the insoluble sheath of dental deposits are the main components of the matrix. This sheath preventing the removal of microorganisms, rather than acid production, is presently considered the major factor responsible for the role of *S. mutans* in damage to tooth enamel [34].

Proteins

The protein composition of EPM has been studied to a considerably lesser degree than its polysaccharide composition, although protein content in some biofilms, such as activated sludge flakes, may exceed their polysaccharide content [35]. *P. aeruginosa* and *B. subtilis* have been best studied in this respect.

Recent studies revealed that many proteins identified in pseudomonad biofilms were associated with membranous vesicles originating from the outer membrane of bacterial cells (OMV). OMV are spherical bubbles with an average diameter of 20 to 200 nm, surrounded by a bilayer phospholipid membrane. They usually contain proteins, lipopolysaccharides, and DNA [36]. They are thought to play important functions in bacterial populations: facilitating intercellular communication, nutrient utilization, and horizontal gene transfer, as well as participation in pathogenesis [37]. While their specific functions in biofilms have not been confirmed unequivocally, their broad occurrence indicates their important biological role [38].

Dozens of proteins were identified in the models of biofilm colonies grown on membrane filters placed on the surface of solid media. They belonged to secreted proteins (such as aminopeptidase), as well as to proteins of cytoplasmic (e.g., ornithine–carbamoyl transferase and arginine deaminase), periplasmic (e.g., TolB), and membrane origin (e.g., porin proteins). Some of them were associated with OMV [39].

For a small number of proteins, such as the *B. subtilis* protein TasA and the amyloid proteins of coiled fimbria (curli), their role in biofilm formation was shown. In the course of biofilm formation, enterobacteria express the curli-specific genes (*csg*) organized in a two-cistron operon, which consists of the *csgDEFG*

and *csgBAC* gene clusters. The *csgEFG* cluster is required for protein export; the CsgD protein is a transcription regulator activating the synthesis of the fimbria structural components encoded by the *csgBA* gene. The RydC protein suppressing expression of the *csgD* gene acts as an additional regulator [40]. Other proteins in EPM (mainly biopolymer hydrolases), which are probably involved in metabolism and in biofilm degradation, play no structural role in EPM.

DNA

Extracellular DNA (eDNA) has been found in biofilms of various microorganisms, such as *P. aeruginosa*, *S. intermedius*, *S. mutans*, *Enterococcus faecalis*, and many species of staphylococci. Autolysis of microbial cells is the main mechanism of its formation. In the case of *S. epidermidis* this was confirmed by the presence of the chromosomal DNA genes from different sites in this molecule [41]. Two types of autolysis were hypothesized: altruistic suicide and fratricidal killing of a part of the population. In the first case (*S. aureus*), the process is similar to apoptosis of eukaryotic cells. In the second case (*E. faecalis*, *B. subtilis*, and *S. pneumoniae*), target and attacking cells are present in a population. The latter kill the target cells with excreted killing factors (this process is analogous to necrosis in eukaryotic cells) [42]. In the case of *P. aeruginosa*, lysis is controlled by the quorum sensing (QS) system. The mutants in this system do not excrete eDNA during the stationary growth phase. This ability is, however, restored on the addition of *N*-acetyl homoserine lactones. Moreover, in wild type strains eDNA secretion is suppressed by furanone, a specific inhibitor of the QS system [43].

The presence of eDNA in biofilms has a stabilizing effect (especially at the early stages of biofilm formation), facilitates adhesion at phase interfaces, and provides for the horizontal transfer of genetic information [44].

FUNCTIONS OF THE EXTRACELLULAR POLYMER MATRIX

EPM is responsible for formation of the three-dimensional biofilm structure whichstabilizes the cells, providing for their contact and protecting them from the hydrodynamic shift forces at the phase interface. EPM probably plays a protective role, increasing microbial resistance to biocides and the agents of the immune system of macroorganisms. We have already mentioned the role of individual EPM components in biofilms. The data concerning their involvement in the processes of biofilm formation and functioning will be summarized in this section [2, 3].

Initial stages of adhesion of microbial cells to biological and abiotic surfaces. Capacity of bacteria for chemotaxis [45] and formation of the surface structures (pili and fimbria) [40] plays an important part at this stage. Polysaccharides, adhesin proteins, extracellular DNA, and amphiphilic compounds (rhamnolipids) are involved in the processes of primary colonization of substrate surfaces [1, 46].

Migration of microbial cells. Transition from reversible to irreversible adhesion of microbial cells is accompanied by their aggregation due to the processes of mutual recognition. Polysaccharides, adhesin proteins, and extracellular DNA participate in these processes [44].

Initial stage of formation of the biofilm phenotype. During this stage, structured EPM is synthesized, while resistance to antibiotics and to the impact of physicochemical factors increases. Polysaccharides, proteins (lectins and amyloids), and extracellular DNA are involved in this process [1].

Completion of formation of the biofilm phenotype. EPM provides protection against desiccation, facilitates accumulation of organic and inorganic compounds, and forms a diffusive barrier, which prevents both the loss of protective compounds and penetration of toxic materials into the biofilm. Almost all EPM components, especially polysaccharides and proteins, are involved in this activity. A new approach to investigation of the dynamics of biofilm formation on solid abiogenic surfaces made it possible to determine that these four stages take 4–6 h, depending on the growth rate of a microorganism [47].

Biofilm maturation and degradation. Maturation of a biofilm is accompanied by formation of complex structures with three-dimensional architecture—including pores and channels within the matrix, similar in shape to myxobacterial fruiting bodies—as well as migration of secondary colonizers into the biofilm. Pores and channels are required for nutrient delivery to the cells. Mature biofilms exhibit genotypic and phenotypic heterogeneity of the cells [1, 48].

Formation of three-dimensional structures depends on a complex system of regulatory processes. Thus, *P. aeruginosa* forms mushroom-like structures with a capped top (in fact, an opening through which motile cells may migrate into the planktonic phase). Rhamnolipids and the *rhlA* gene are involved in cap formation.

The cells may be released from the biofilm as planktonic forms for further propagation. One of the factors regulating this process is c-di-GMP. Its decreased level results in transition of the cells from the biofilm to a planktonic form. In some (though not in all) species, local cell lysis in some sites of the biofilm precedes active transition to the planktonic form. Lysed cells provide nutrients for the planktonic ones. In the cells released into the medium, the expression of some genes changes: the genes of matrix synthesis are suppressed, while the genes responsible for motility and chemotaxis are expressed [48].

Carbon limitation may be an external factor promoting cell transition to planktonic forms. Hypoxia also increases the number of planktonic cells [49].

The mature EPM acts as a trap for extracellular enzymes hydrolyzing exogenous macromolecules and as a factor stabilizing these enzymes. The low-molecular mass products of hydrolysis serve as nutrient substrates for the microorganisms inside the biofilm. The EPM components (proteins, polysaccharides, and lipids) may also be used as nutrient sources. EPM protects microbial cells from biocides and extreme environmental impacts. In *Burkholderia* biofilms, the EPM polysaccharides were shown to play an important role in these processes [50]. For some bacteria, impaired synthesis of acidic polysaccharides was shown to increase the sensitivity of their biofilms to such impacts [51–53].

EPM provides favorable conditions for exchange of genetic information and horizontal gene transfer. The rate of these processes in biofilms is an order of magnitude higher than in planktonic microbial cultures, partially due to increased stability of extrachromosomal genetic elements and a broader host spectrum [54].

Since in natural conditions a biofilm provides not only for protection and preservation of microbial populations, but also for their propagation, the mechanisms of dispersion releasing planktonic cells into the environment may be activated at a certain stage of its development. These mechanisms will be discussed in detail in the following section.

REGULATION OF EPM FORMATION

Regulation of the biosynthesis of EPM components has been studied in most detail for pseudomonads. Three major types of regulatory systems employing c-di-GMP, QS components, and small RNA (sRNA) were shown to participate in these processes [55].

Cyclic diguanosine monophosphate. High c-di-GMP levels induce the synthesis of adhesins and polysaccharide EPM components and thus to initiation of biofilm formation. On the contrary, decreased c-di-GMP level initiates dispersion of the biofilm with release and spreading of planktonic cells. The level of c-di-GMP is maintained by coordinated operation of two types of key enzymes, diguanylate cyclases (DGC) and phosphodiesterases (PDEs). Activity of these enzymes is regulated by the signals from the environment [56]. In the case of *P. aeruginosa*, molecular mechanisms of c-di-GMP-dependent regulation have been analyzed in detail. Phosphorylation of the WspR component of the chemotaxis regulatory system was observed in response to the interaction of a cell with a phase interface; this modification stimulated c-di-GMP synthesis [57].

Apart from biosynthesis of polysaccharides (including cellulose), c-di-GMP regulates formation of the LapA protein in *P. putida*. This adhesin protein is located at the surface of bacterial cells and plays a major role in biofilm formation. Thus, a *P. putida* with inactivated genes encoding the synthesis of all polysaccharides was nevertheless capable of biofilm formation in a flow system [58]. Localization of LapA at the surface of bacterial cells is regulated by LapG proteinase, which may affect release of this protein from the cells by cleaving its N-terminal part. Activity of the LapG proteinase is, in turn, modulated by the LapD protein of the cytoplasmic membrane [59].

In *P. aeruginosa*, an interesting mechanism for upregulation of the biosynthesis of the EPM polysaccharides was revealed, which resembled autocrine signaling in eukaryotes. Psl, the major EPM polysaccharide, acts as a signal stimulating diguanylate cyclases, SiaD and SadC, which results in elevated c-di-GMP levels. In turn, c-di-GMP enhances the synthesis of Psl and other components of the biofilm EPM [60].

External factors affecting PDE activity and therefore c-di-GMP and biofilm dispersion—such as starvation, hypoxia, and nitric oxide—attract attention of researchers. Although molecular mechanisms of these effects have not been accurately determined, these approaches to PDE activation are considered promising for counteracting undesired biofilm formation [61].

Quorum sensing systems. At least three QS systems are functioning in pseudomonads. LasIR and RhlIR, the best-studied systems, use *N*-acyl homoserine lactones (AHL) as signal molecules. AQ, the third system, is alkylquinolone-dependent [62, 63]. For these systems participation in the processes of biofilm formation was established.

Over 70 bacterial genera (such *Alpha-*, *Beta-*, and *Gammaproteobacteria* as *Aeromonas*, *Agrobacterium*, *Burkholderia*, *Chromobacterium*, *Citrobacter*, *Enterobacter*, *Erwinia*, *Hafnia*, *Nitrosomonas*, *Obesumbacterium*, *Pantoea*, *Pseudomonas*, *Rahnella*, *Ralstonia*, *Rhodobacter*, *Rhizobium*, *Serratia*, *Vibrio*, *Xenorhabdus*, and *Yersinia*) are know to utilize the QS system based on AHL, which are termed type I autoinducers (AI-1). The critical AHL concentration, which correlates with the density of a bacterial population, controls expression of the genes, including those associated with biofilm formation. A typical AHL-dependent system consists of three components: a molecule of a homoserine lactone derivative, a LuxI-like signal synthase, and a LuxR-like signal receptor. In some cases the system also contains special membrane pumps for transporting long-chain homoserine lactones from the medium. *E. coli* cells contain an SdiA receptor, which may bind to AHL arriving from other bacteria within a given ecosystem. After AHL binding with the receptor, the conformation of this protein changes to enable its interaction with DNA and launch the transcription of QS-dependent genes, including *luxI*. This results in an avalanche-like increase in the concentration of signal molecules in the medium.

While the role of AHL-dependent QS systems in biofilm formation has been shown in many works, molecular mechanisms of these processes remain unknown. Mutation of the *cviI* gene encoding AHL synthase in *Chromobacterium violaceum* was recently shown to result in impaired synthesis of the polysaccharide components of the biofilm EPM, which is accompanied by an increase of the biofilm sensitivity to extreme environmental impacts and to antibiotics [51, 53].

Moreover, the AHL-dependent QS system participates in biofilm formation by regulating the synthesis of siderophores and lectin proteins, as well as by generating eDNA and rhamnolipids; the role of the latter was discussed above.

Subsidiary QS systems, such as the AQ system dependent on alkyl quinolones (4-hydroxy-2-heptyl, 4-hydroxy-2-nonil, and 3,4-dihydroxy-2-heptylquinolones), are common in many gram-negative bacteria, such as *Pseudomonas*, *Citrobacter*, and *Burkholderia* spp. [64]. These autoregulators were shown to induce the *lecA* gene encoding the lectin responsible for stable biofilm architecture in *P. aeruginosa* PAO1.

No convincing data are available concerning the role of other QS systems, such as the systems using AI-2 and AI-3 autoinducers, or peptide compounds in gram-positive bacteria, in biofilm formation.

Small RNA. A number of processes associated with bacterial motility, transition to a "sedentary" mode, and biofilm formation are directly or indirectly regulated by sRNA [65]. These are polyribonucleotides 80–200 bp long, which interact either directly with the messenger RNA or indirectly via regulatory molecules. Downregulating small RNAs interact with ribosomes by binding with the Shine–Dalgarno sequence on mRNA or cause mRNA degradation by initiating the assemblage of RNase E. Most of the studied sRNA interact with the 5' nontranslated mRNA region or with an open reading frame. Upregulating sRNA unwind the mRNA secondary structures, blocking the Shine–Dalgarno sequence or restructuring the 5' nontranslated regions to open the binding sites for ribosomes upstream along the sequence [66, 67].

In *E. coli* and *Salmonella thyphimurium*, sRNA regulate the activation and suppression of the regulatory cascade of flagella synthesis controlled by the *fhlDC* operon. McaS (multi-cellular adhesive sRNA) directly binds two sites in the 5' nontranslated region. Along an as yet unknown pathway, MicA (the mRNA regulator read from the *ompA* gene) suppresses porin synthesis. Overexpression of McaS and MicA results in enhanced cell motility [68]. ArcZ (*arc*-associated sRNA), OmrA (OmpR regulated sRNA A), OmrB (OmpR regulated sRNA B), OxyS (oxidative stress-related), SdsR (sigma *S*-dependent sRNA), and GadY (*gad* gene-related sRNA) suppress *fhlDC* using various mechanisms. The first four interact complementarily with mRNA, partially overlapping the Shine–Dalgarno sequence and thus blocking translation [68]. GadY and SdsR act indirectly, via the regulation of expression of other genes [65].

In *E. coli* the reaction cascade responsible for the synthesis of coiled fimbria (curli) and including the genes *rpoS*, *ydaM*, and *csgD* is also regulated by sRNA. The σ^S factor encoded by *rpoS* is a global regulator of over 500 genes of the stationary phase and of stress-induced genes. In *S. thyphimurium*, σ^S is regulated by three sRNA: DsrA, RprA, and ArcZ [65]. DsrA (downstream region of *rcsA*) is expressed at low temperatures, probably causing an increase of the σ^S concentration under cold conditions. The physiological role of RprA (RpoS regulator A) in the *rpoS* regulation is still unclear. In *E. coli*, *csgD* expression is directly suppressed by McaS, RprA, and OmrA, although direct interaction of the *csgD* product with mRNA has not been demonstrated [67]. A recently published article summarized all the data on sRNA action in a detailed scheme [40].

In pseudomonads, sRNA also play an important role in the processes of biofilm formation [55]. In *P. aeruginosa*, deletion of the *retS* gene—a component of a regulatory system including three sensor kinases, RetS, GacS, and Lad—results in decreased capacity for biofilm formation. The GacS kinase phosphorylates the GacA protein; in this modification, it activates the synthesis of two sRNA: RsmZ and RsmY. The latter may bind to the RsmA protein, suppressing its ability to inhibit the genes responsible for the synthesis of the Psl polysaccharide and for the functioning of the QS system [69]. In *P. putida* and *P. fluorescens*, sRNA carry out their regulatory functions via the GacAS regulatory system involved in biofilm formation and synthesis of secondary metabolites. The GacAS system carries out upregulation of expression of the genes involved in polysaccharide synthesis and in the c-di-GMP-dependent processes [70].

Indole derivatives. Indoles are universal intercellular signal molecules in various bacteria, acting as QS systems and responsible for direct control of a number of processes, including those responsible for antibiotic resistance, virulence, and biofilm formation [71]. Over 80 bacteria species were shown to produce indoles. Suppression of biofilm formation by high extracellular indole concentration (over 600 μM) accumulating in *E. coli* cultures did not result from indole toxicity. On the other hand, introduction of indole to the cultures of *P. aeruginosa* and *P. fluorescens*, which do not produce indole, stimulated biofilm formation [72].

Small cell wall-modifying molecules. In gram-positive bacteria, peptide bridges between the cell wall peptidoglycan molecules are probably capable of providing intercellular contacts in biofilms. Bacteria (e.g., *S. aureus*) synthesize D-amino acids: D-tyrosine, D-leucine, D-tryptophan, and D-methionine, which are able to degrade existing biofilms and prevent formation of new ones [73]. When incorporated into the muramopeptide in the fourth or fifth position in the peptide chain, these noncanonical D-amino acids,

which are not the usual components of peptidoglycan, interact with the anchor protein of the amyloid fimbria involved in fastening the biofilm structure. *B. subtilis* produces noncanonical D-amino acids at the stage of mature biofilms. Degradation of the biofilm structure by D-amino acids results from delocalization of the cell wall protein YqxM/TapA, which acts as an anchor for amyloid fimbria, but is not related to their cytotoxicity [74]. The cell membrane may also act as a target for signal molecules, such as zaragozic acid, which is probably involved in biofilm degradation [75].

Molecules affecting the matrix. Apart from biopolymers, including enzymes (polysaccharide hydrolases, nucleases, and proteases) and global regulators (AI-1, c-di-GMP), some small molecules are involved in the regulation of the composition of the biofilm matrix [75]. In *B. subtilis*, polyamines structurally similar to norspermidine specifically attack the polysaccharide component of the matrix, binding to the negatively charged groups by electrostatic interaction and hydrogen bonds. A family of norspermidine-like compounds degrading the polymer matrix of *B. subtilis* and *S. aureus* biofilms was recently synthesized [76]. Spermidines, however, play a dual role and in some cases may stimulate biofilm growth, probably acting as signal molecule [77]. Affinity of parthenolide (a sesquiterpene lactone with anti-inflammatory and antitumor activity [78]), an inhibitor of biofilm growth, to the protein component of the matrix was demonstrated for *B. cereus*, *B. subtilis*, and *E. coli*. This compound interacts with the fimbria TasA protein, preventing fimbria assembly and therefore inhibiting biofilm growth [79]. Phenol-soluble modulins, the peptide which may either decrease the surface tension and thus stimulate biofilm degradation or assemble to fimbria and form the structure of the matrix, also exhibit dual action [80].

Role of the processes of programmed cell death (apoptosis). Programmed cell death (PCD) is mostly a feature of multicellular eukaryotic organisms. However, a number of prokaryotic systems resulting in PCD have been described. It was shown previously that lysis of a specific fraction of the cell population was required for formation of multicellular fruiting bodies by *Myxococcus xanthus* [81]. The PCD system associated with synthesis of NO, the molecule regulating both PCD and biofilm degradation in *V. cholera*, is of interest [82]. Association between NO and PCD was also found in *B. subtilis* [83]. Low, nontoxic concentrations of exogenous NO stimulate biofilm degradation and cell motility in *P. aeruginosa*. In mature *P. aeruginosa* biofilms, the processes of PCD and biofilm degradation are associated with accumulation of the oxidative and nitroactive stress [75]. Apart from its role in PCD and in formation of cavities within *P. aeruginosa* biofilms, NO also modulates the level of c-di-GMP, thus stimulating cell motility [84].

The role of the regulatory mechanisms occurring in the biofilm microbial populations in microevolution and adaptive mutagenesis was discussed in recent reviews [85, 86].

ACKNOWLEDGMENTS

The work was supported by the Russian Foundation for Basic Research, project no. 13-04-01145.

REFERENCES

1. Nikolaev, Yu.A. and Plakunov, V.K., Biofilm—"City of microbes" or an analogue of multicellular organisms?, *Microbiology* (Moscow), 2007, vol. 76, no. 2, pp. 125–138.
2. Flemming, H. and Wingender, J., The biofilm matrix, *Nature Rev. Microbiol.*, 2010, vol. 8, pp. 623–633.
3. Flemming, H.-C., The perfect slime, *Colloids Surf.*, 2011, vol. 86, pp. 251–259.
4. Knirel, Y.A., Bystrova, O.V., Kocharova, N.A., Zähringer, U., and Pier, G., Review. Conserved and variable structural features in the lipopolysaccharide of *Pseudomonas, J. Endotoxin Res.*, 2006, vol. 12, pp. 324–336.
5. Kocincova, D. and Lam, J.S., Structural diversity of the core oligosaccharide domain of *Pseudomonas aeruginosa* lipopolysaccharide, *Biochemistry* (Moscow), 2011, vol. 76, pp. 755–760.
6. Ramsey, D.M. and Wozniak, D.J., Understanding the control of *Pseudomonas aeruginosa* alginate synthesis and the prospects for management of chronic infections in cystic fibrosis, *Mol. Microbiol.*, 2005, vol. 56, pp. 309–322.
7. Branda, S.S., Vik, A., Friedman, L., and Kolter, R., Biofilms: the matrix revisited, *Trends Microbiol.*, 2005, vol. 13, pp. 20–26.
8. Mann, E.E. and Wozniak, D.J., *Pseudomonas* biofilm matrix composition and niche biology, *FEMS Microbiol. Rev.,* 2012, vol. 36, pp. 893–916.
9. Wozniak, D.J., Wyckoff, T.J.O., Starkey, M., Keyser, R., Azadi, P., O'Toole, G.A., and Parsek, M.R., Alginate is not a significant component of the extracellular polysaccharide matrix of PA14 and PAO1 *Pseudomonas aeruginosa* biofilms, *Proc. Natl. Acad. Sci. U. S. A.*, 2003, vol. 100, pp. 7907–7912.
10. Orgad, O., Oren, Y., Walker, S.L., and Herzberg, M., The role of alginate in *Pseudomonas aeruginosa* EPS adherence, viscoelastic properties and cell attachment, *Biofouling*, 2011, vol. 27, pp. 787–798.
11. Wiens, J.R., Vasil, A.I., Schurr, M.J., and Vasil, M.L., Iron-regulated expression of alginate production, mucoid phenotype, and biofilm formation by *Pseudomonas aeruginosa, MBio*, 2014, vol. 5, no. 1. http://journals.asm.org/subscriptions
12. Lee, K., Lim, E.J., Kim, K.S., Huang, S-L., Veeranagouda, Y., and Rehm, B.H.A., An alginate-like exopolysaccharide biosynthesis gene cluster involved in biofilm aerial structure formation by *Pseudomonas alkylphenolia*, *Appl. Microbiol. Biotechnol.*, 2014, vol. 98, pp. 4137–4148. http://link.springer.com/article/10.1007%2Fs00253-014-5529-6
13. Pagès, D., Sanchez, L., Conrod, S., Gidrol, X., Fekete, A., Schmitt-Kopplin, P., Heulin, T., and Ach-

ouak, W., Exploration of intraclonal adaptation mechanisms of *Pseudomonas brassicacearum* facing cadmium toxicity, *Environ. Microbiol.*, 2007, vol. 9, pp. 2820–2835.

14. Ma, L., Conover, M., Lu, H., Parsek, M.R., Bayles, K., and Wozniak, D.J., Assembly and development of the *Pseudomonas aeruginosa* biofilm matrix, *PLoS Pathogens*, 2009, vol. 5, no. 3. e1000354.6. www.plospathogens.org
15. Byrd, M.S., Sadovskaya, I., Vinogradov, E., Lu, H., Sprinkle, A.B., Richardson, S.H., Ma, L., Ralston, B., Parsek, M.R., Anderson, E.M., Lam, J.S., and Wozniak, D.J., Genetic and biochemical analyses of the *Pseudomonas aeruginosa* Psl exopolysaccharide reveal overlapping roles for polysaccharide synthesis enzymes in Psl and LPS production, *Mol. Microbiol.*, 2009, vol. 73, pp. 622–638.
16. Irie, Y., Starkey, M., Edwards, A.N., Wozniak, D.J., Romeo, T., and Parsek, M.R., *Pseudomonas aeruginosa* posttranscriptional regulator RsmA represses biofilm extracellular polysaccharide Psl synthesis, *Mol. Microbiol.*, 2010, vol. 78, pp. 158–172.
17. Franklin, M.J., Nivens, D.E., Weadge, J.T., and Howell, P.L., Biosynthesis of the *Pseudomonas aeruginosa* extracellular polysaccharides, alginate, Pel, and Psl, *Front. Microbiol.*, 2011, vol. 2. A 167. www.frontiersin.org
18. Colvin, K.M, Gordon, V.D., Murakami, K., Borlee, B.R., Wozniak, D.J., Wong, G.C.L., and Parsek, M.R., The Pel polysaccharide can serve a structural and protective role in the biofilm matrix of *Pseudomonas aeruginosa*, *PLoS Pathog.*, 2011, vol. 7. E.1. e001264. www.plospathogens.org
19. Ghafoor, A., Hay, I.D., and Rehm, B.H.A., Role of exopolysaccharides in *Pseudomonas aeruginosa* biofilm formation and architecture, *Appl. Environ. Microbiol.*, 2011, vol. 77, pp. 5238–5246.
20. Cooley, B.J., Thatcher, T.W., Hashmi, S.M., L'Her, G., Le, H.H., Hurwitz, D.A., Provenzano, D., Touhami, A., and Gordon, V.D., The extracellular polysaccharide Pel makes the attachment of *P. aeruginosa* to surfaces symmetric and short-ranged, *Soft Matter*, 2013, vol. 9, pp. 3871–3876.
21. Serra, D.O., Richter, A.M., Klauck, G., Mika, F., and Hengge, R., Microanatomy at cellular resolution and spatial order of physiological differentiation in a bacterial biofilm, *Mbio. Asm. org.*, 2013, vol. 4 I.2. e00103-13
22. Spiers, A.J. and Rainey, P.B., The *Pseudomonas fluorescens* SBW25 wrinkley spreader biofilm requires attachment factor, cellulose fibre and LPS interactions to maintain strength and integrity, *Microbiology* (UK), 2005, vol. 151, pp. 2829–2839.
23. Nielsen, L, Li, X. and Halverson, L.J., Cell–cell and cell–surface interactions mediated by cellulose and a novel exopolysaccharide contribute to *Pseudomonas putida* biofilm formation and fitness under water-limiting conditions, *Environ. Microbiol.*, 2011, vol. 13, pp. 1342–1356.
24. Morgan, J.L., McNamara, J.T., and Zimmer, J., Mechanism of activation of bacterial cellulose synthase by cyclic di-GMP, *Nature Structur. Molec. Biol.*, 2014, vol. 21, pp. 489–498.
25. Abdel-Mawgoud, A.M., Lepine, F., and Deziel, E., Rhamnolipids: diversity of structures, microbial origins and roles, *Appl. Microbiol. Biotechnol.,* 2010, vol. 86, pp. 1323–1336.
26. Dubeau, D., Deziel, E., Woods, D.E., and Lepine, F., *Burkholderia thailandensis* harbors two identical *rhl* gene clusters responsible for the biosynthesis of rhamnolipids, *BMC Microbiol.*, 2009, vol. 9, p. 263. www.biomedcentral.com/1471-2180/9/263
27. Müller, M.M., Kügler, J.H., Henkel, M., Gerlitzki, M., Hörmann, B., Pöhnlein, M., Syldatk, C., and Hausmann, R., Rhamnolipids–next generation surfactants?, *J. Biotechnol.*, 2012, vol. 162, pp. 366–380.
28. Lopez, D., Vlamakis, H., and Kolter, R., Biofilms, *Cold Spring Harb. Perspect. Biol.*, 2010. V. 2. a000398
29. Marvasi, M., Visscher, P.T., and Martinez, L.C. Exopolymeric substances (EPS) from *Bacillus subtilis*: polymers and genes encoding their synthesis, *FEMS Microbiol. Lett.*, 2010, vol. 313, pp. 1–9.
30. Branda, S.S., Gonzalez-Pastor, J. E., Dervyn, E., Ehrlich, S.D., Losick, R., and Kolter, R., Genes involved in formation of structured multicellular communities by *Bacillus subtilis, J. Bacteriol.*, 2004, vol. 186, pp. 3970–3979.
31. Cue, D., Lei, M.G., and Lee, C.Y., Activation of sarX by Rbf is required for biofilm formation and icaADBC expression in *Staphylococcus aureus, J. Bacteriol.*, 2013, vol. 195, pp. 1515–524.
32. Lembré, P., Vendrely, C., and Di Martino, P., Identification of an amyloidogenic peptide from the Bap protein of *Staphylococcus epidermidis*, *Protein Peptide Lett.*, 2014, vol. 21, pp. 75–79.
33. Romero, D., Vlamakis, H., Losick, R., and Kolter, R., Functional analysis of the accessory protein TapA in *Bacillus subtilis* amyloid fiber assembly, *J. Bacteriol.*, 2014, vol. 196, pp. 1505–1513.
34. Koo, H., Falsetta, M.L., and Klein, M.I., The exopolysaccharide matrix: a virulence determinant of cariogenic biofilm, *J. Dent. Res.*, 2013, vol. 92, pp. 1065–1073.
35. McSwain, B.S., Irvine, R.L., Hausner, M., and Wilderer, P.A., Composition and distribution of extracellular polymeric substances in aerobic flocs and granular sludge, *Appl. Environ. Microbiol.*, 2005, vol. 71, pp. 1051–1057.
36. Kulp, A. and Kuehn, M.J., Biological functions and biogenesis of secreted bacterial outer membrane vesicles, *Annu. Rev. Microbiol.*, 2010, vol. 64, pp. 163–184.
37. Tashiro, Y., Uchiyama, H., and Nomura, N., Multifunctional membrane vesicles in *Pseudomonas aeruginosa*, *Environ. Microbiol.*, 2012, vol. 14, pp. 1349–1362.
38. Schooling, S.R. and Beveridge, T.J., Membrane vesicles: an overlooked component of the matrices of biofilms, *J. Bacteriol.*, 2006, vol. 188, pp. 5945–5957.
39. Toyofuku, M., Roschitzki, B., Riedel, K., and Eberl, L., Identification of proteins associated with the *Pseudomonas aeruginosa* biofilm extracellular matrix, *J. Proteom. Res.*, 2012, vol. 11, pp. 4906–4915.
40. Bordeau, V. and Felden, B., Curli synthesis and biofilm formation in enteric bacteria are controlled by a dynamic small RNA module made up of a pseudoknot

assisted by an RNA chaperone, *Nucleic Acids Res.*, 2014, vol. 42, pp. 4682–4696.

41. Qin, Z., Ou, Y., Yang, L., Zhu, Y., Tolker-Nielsen, T., Molin, S., and Qu, D., Role of autolysin-mediated DNA release in biofilm formation of *Staphylococcus epidermidis*, *Microbiology* (UK), 2007, vol. 153, pp. 2083–2092.
42. Thomas, V.C. and Hancock, L.E., Suicide and fratricide in bacterial biofilms, *Int. J. Artif. Organs*, 2009, vol.32, pp. 537–544.
43. Allesen-Holm, M., Barken, K.B., Yang, L., Klausen, M., Webb, J.S., Kjelleberg, S., Molin, S., Givskov, M., and Tolker-Nielsen, T., A characterization of DNA release in *Pseudomonas aeruginosa* cultures and biofilms, *Mol. Microbiol.*, 2006, vol. 59, pp. 1114–1128.
44. Okshevsky, M. and Meyer, R.L., The role of extracellular DNA in the establishment, maintenance and perpetuation of bacterial biofilms, *Crit. Rev. Microbiol.*, 2013. Informa Healthcare USA http://informahealthcare.com/mby
45. Schmidt, J., Musken, M., Becker, T., Magnowska, Z., Bertinetti, D., Moller, S., Zimmermann, B., Herberg, F. W., Jansch, L., and Haussler, S., The *Pseudomonas aeruginosa* chemotaxis methyltransferase CheR1 impacts on bacterial surface sampling, *PLoS One*, 2011, vol. 6. I.3. e18184
46. Nickzad, A. and Deziel, E., The involvement of rhamnolipids in microbial cell adhesion and biofilm development—an approach for control?, *Lett. Appl. Microbiol.*, 2014, vol. 58, pp. 447–453.
47. Strelkova, E.A., Zhurina, M.V., Plakunov, V.K., and Belyaev, S.S., Stimulation of biofilm formation by antibiotics, *Microbiology* (Moscow), 2012, vol. 81, no. 2, pp. 259–262.
48. Haussler, S. and Fuqua, C., Biofilms: new discoveries and significant wrinkles in a dynamic field, *J. Bacteriol.*, 2012, vol. 195, pp. 2947–2958.
49. An, S., Wu, J., and Zhang, L.H., Modulation of *Pseudomonas aeruginosa* biofilm dispersal by a cyclic-di-GMP phosphodiesterase with a putative hypoxia-sensing domain, *Appl. Environ. Microbiol.*, 2010, vol. 76, pp. 8160–8173.
50. Ferreira, A.S., Silva, I.N., Oliveira, V.H., Cunha, R., and Moreira, L.M., Insights into the role of extracellular polysaccharides in *Burkholderia* adaptation to different environments, *Front. Cell. Infect. Microbiol.*, 2011, vol. 1 A. 16. www.frontiersin.org
51. Strelkova, E.A., Pozdnyakova, N.V., Zhurina, M.V., Plakunov, V.K., and Belyaev, S.S., Role of the extracellular polymer matrix in resistance of bacterial biofilms to extreme environmental factors, *Microbiology* (Moscow), 2013, vol. 82, no. 2, pp. 119–125.
52. Zhurina, M.V., Kostrikina, N.A., Parshina, E.Yu., Strelkova, E.A., Yusipovich, A.I., Maksimov, G.V., and Plakunov, V.K., Visualization of the extracellular polymeric matrix of *Chromobacterium violaceum* biofilms by microscopic methods, *Microbiology* (Moscow), 2013, vol. 82, no. 4, pp. 517–524.
53. Mart'yanov, S.V., Zhurina, M.V., El'-Registan, G.I., and Plakunov, V.K., Activation of formation of bacterial biofilms by azithromycin and countering this effect, *Microbiology* (Moscow), 2014, vol. 83, no. 6, pp. 723–731.
54. Madsen, J.S., Burmølle, M., Hansen, L.H., and Sørensen, S.J., The interconnection between biofilm formation and horizontal gene transfer, *FEMS Immunol. Med. Microbiol.*, 2012, vol. 65, pp. 183–195.
55. Fazli, M., Almblad, H., Rybtke, M. L., Givskov, M., Eberl, L., and Tolker-Nielsen, T., Regulation of biofilm formation in *Pseudomonas* and *Burkholderia* species, *Environ. Microbiol.*, 2014. doi: 10.1111/1462-2920.12448
56. Römling, U., Galperin, M.Y., and Gomelsky, M., Cyclic di-GMP: the first 25 years of a universal bacterial second messenger, *Microbiol. Mol. Biol. Rev.*, 2013, vol. 77, pp. 1–52.
57. O'Connor, J.R., Kuwada, N.J., Huangyutitham, V., Wiggins, P.A., and Harwood, C.S. Surface sensing and lateral subcellular localization of WspA, the receptor in a chemosensory-like system leading to c-di-GMP production, *Mol. Microbiol.*, 2012, vol. 86, pp. 720–729.
58. Nilsson, M., Chiang, W.C., Fazli, M., Gjermansen, M., Givskov, M., and Tolker-Nielsen, T., Influence of putative exopolysaccharide genes on *Pseudomonas putida* KT2440 biofilm stability, *Environ. Microbiol.*, 2011, vol. 13, pp. 1357–1369.
59. Newell, P.D., Boyd, C.D., Sondermann, H., and O'Toole, G.A., A c-di-GMP effector system controls cell adhesion by inside-out signaling and surface protein cleavage, *PLoS Biol.*, 2011, vol. 9. e1000587
60. Irie, Y., Borlee, B.R., O'Connor, J.R., Hill, P.J., Harwood, C.S., Wozniak, D.J., and Parsek, M.R., Self-produced exopolysaccharide is a signal that stimulates biofilm formation in *Pseudomonas aeruginosa*, *Proc. Natl. Acad. Sci. U. S. A.*, 2012, vol. 109, no. 50. www.pnas.org/cgi/doi/10.1073/pnas.1217993109
61. Li, Y., Heine, S., Entian, M., Sauer, K., and Frankenberg-Dinkel, N., NO-Induced biofilm dispersion in *Pseudomonas aeruginosa* is mediated by an MHYT domain-coupled phosphodiesterase, *J. Bacteriol.*, 2013, vol. 195, pp. 3531–3542.
62. Khmel', I.A., Quorum-sensing regulation of gene expression: fundamental and applied aspects and the role in bacterial communication, *Microbiology* (Moscow), 2006, vol. 75, no. 4, pp. 390–397.
63. Li, Z. and Nair, S.K., Quorum sensing: how bacteria can coordinate activity and synchronize their response to external signals?, *Protein Sci.*, 2012, vol. 21, pp. 1403–1417.
64. Gupta, R. and Schuster, M., Quorum sensing modulates colony morphology through alkyl quinolones in *Pseudomonas aeruginosa*, *BMC Microbiol.*, 2012, vol. 12, no. 30. www.biomedcentral.com/1471-2180/12/30
65. Mika, F. and Hengge, R., Small regulatory RNAs in the control of motility and biofilm formation in *E. coli* and *Salmonella, Int. J. Mol. Sci.*, 2013, vol. 14, pp. 4560–4579.
66. Thomason, M.K., Fontaine, F., De Lay, N., and Storz, G., A small RNA that regulates motility and biofilm formation in response to changes in nutrient availability in *Escherichia coli, Mol. Microbiol.*, 2012, vol. 84, pp. 17–35.

67. Jørgensen, M.G., Nielsen, J.S., Boysen, A., Franch, T., Møller-Jensen, J., and Valentin-Hansen, P., Small regulatory RNAs control the multi-cellular adhesive lifestyle of *Escherichia coli, Mol. Microbiol.*, 2012, vol. 84, pp. 36–50.

68. De Lay, N. and Gottesman, S., A complex network of small non-coding RNAs regulate motility in *Escherichia coli, Mol. Microbiol.*, 2012, vol. 86, pp. 524–538.

69. Marden, J.N., Diaz, M.R., Walton, W.G., Gode, C.J., Betts, L., and Urbanowski, M.L., An unusual CsrA family member operates in series with RsmA to amplify posttranscriptional responses in *Pseudomonas aeruginosa, Proc. Natl. Acad. Sci. U. S. A.*, 2013, vol. 110, pp. 15055–15060.

70. Duque, E., de la Torre, J., Bernal, P., Molina-Henares, M.A., Alaminos, M., and Espinosa-Urgel, M., Identification of reciprocal adhesion genes in pathogenic and non-pathogenic *Pseudomonas, Environ. Microbiol.*, 2013, vol. 15, pp. 36–48.

71. Wood, T.K., Insights on *Escherichia coli* biofilm formation and inhibition from whole-transcriptome profiling, *Environ. Microbiol.*, 2009, vol. 11, pp. 1–15.

72. Lee, J., Jayaraman, A., and Wood, T.K., Indole is an inter-species biofilm signal mediated by SdiA, *BMC Microbiol.*, 2007. 7:42. http://www.biomedcentral.com/1471-2180/7/42.

73. Hochbaum, A.I., Kolodkin-Gal, I., and Foulston, L., Inhibitory effects of D-amino acids on *Staphylococcus aureus* biofilm development, *J. Bacteriol.*, 2011, vol. 193, pp. 5616–5622.

74. Romero, D., Vlamakis, H., and Losick, R., An accessory protein required for anchoring and assembly of amyloid fibres in *B. subtilis* biofilms, *Mol. Microbiol.*, 2011, vol. 80, pp. 1155–1168.

75. Oppenheimer-Shaanan, Y., Steinberg N., and Kolodkin-Gal, I., Small molecules are natural triggers for the disassembly of biofilms, *Trends Microbiol.*, 2013, vol. 11, pp. 594–601.

76. Bottcher, T., Kolodkin-Gal, I., and Kolter, R., Synthesis and activity of biomimetic biofilm disruptors, *J. Am. Chem. Soc.*, 2013, vol. 135, pp. 2927–2930.

77. Burrell, M., Hanfrey, C.C., Murray, E.J., Stanley-Wall, N.R., and Michael, A.J., Evolution and multiplicity of arginine decarboxylases in polyamine biosynthesis and essential role in *Bacillus subtilis* biofilm formation, *J. Biol. Chem.*, 2010, vol. 285, pp. 39224–39238.

78. Mathema, V.B., Koh, Y.S., and Thakuri, B.C., Parthenolide, a sesquiterpene lactone, expresses multiple anti-cancer and anti-inflammatory activities, *Inflammation*, 2012, vol. 35, pp. 560–565.

79. Romero, D., Sanabria-Valentín, E., and Vlamakis, H., Biofilm inhibitors that target amyloid proteins, *Chem. Biol.*, 2013, vol. 20, pp. 102–110.

80. Schwartz, K., Syed, A.K., Stephenson, R.E., Rickard, A.H., and Boles, B.R., Functional amyloids composed of phenol soluble modulins stabilize *Staphylococcus aureus* biofilms, *PLoS Pathog.*, 2012, vol. 8(6): e1002744

81. Rosenbluh, A. and Rosenberg, E., 1990. Role of autocide AMI in development of *Myxococcus xanthus*, *J. Bacteriol.*, vol. 172, pp. 4307–4314.

82. Karatan, E., Duncan, T.R., and Watnick, P.I., NspS, a predicted polyamine sensor, mediates activation of *Vibrio cholerae* biofilm formation by norspermidine, *J. Bacteriol.*, 2005, vol. 187, pp. 7434–7443.

83. Schreiber, F., Beutler, M., and Enning, D., The role of nitric-oxide-synthase-derived nitric oxide in multicellular traits of *Bacillus subtilis* 3610: biofilm formation, swarming, and dispersal, *BMC Microbiol.*, 2011, vol. 11:111. doi: 10.1186/1471-2180-11-111

84. Li, Y., Heine, S., and Entian, M., NO-induced biofilm dispersion in *Pseudomonas aeruginosa* is mediated by a MHYT-domain coupled phosphodiesterase, *J. Bacteriol.*, 2013, vol. 195, pp. 3531–3542.

85. Plakunov, V.K., Strelkova, E.A., and Zhurina, M.V., Persistence and adaptive mutagenesis in biofilms, *Microbiology* (Moscow), 2010, vol. 79, no. 4, pp. 424–434.

86. Ryall, B., Eydallin, G., and Ferenci, T., Culture history and population heterogeneity as determinants of bacterial adaptation: the adaptomics of a single environmental transition, *Microbiol. Mol. Biol. Rev.*, 2012, vol. 76, pp. 597–625.

Translated by P. Sigalevich