REVIEWS

Response of Bacteria to Mechanical Stimuli

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Received April 1, 2021; revised May 2, 2021; accepted May 9, 2021

Abstract—Bacteria adapt rapidly to changes in ambient conditions, constantly inspecting their surroundings by means of their sensor systems. These systems are often thought to respond only to signals of a chemical nature. Yet, bacteria are often affected by mechanical forces, e.g., during transition from planktonic to sessile state. Mechanical stimuli, however, have seldom been considered as the signals bacteria can sense and respond to. Nonetheless, bacteria perceive mechanical stimuli, generate signals, and develop responses. This review analyzes the information on the way bacteria respond to mechanical stimuli and outlines how bacteria convert incoming signals into appropriate responses.

Keywords: bacteria, mechanosensitivity, mechanotransduction, motility, biofilms, swarming, *Azospirillum*, *Bacillus*, *Proteus*, *Pseudomonas*

DOI: 10.1134/S0026261721050052

MECHANICAL PROPERTIES OF THE ENVIRONMENT AFFECT THE BEHAVIOR OF BACTERIA

Microorganisms can colonize diverse environments, which are characterized by heterogeneity and dynamism of their physicochemical and mechanical properties (Dufrêne and Persat, 2020). Bacteria adapt rapidly to changes in ambient conditions, constantly inspecting their surroundings by means of their sensor systems (Berg, 1975; Bible et al., 2008; Vladimirov and Sourjik, 2009; Chawla et al., 2020; Fajardo-Cavazos and Nicholson, 2021). Free-moving (planktonic) bacteria in liquid media interact with multiple factors. These may be abiotic parameters of the environment, other microorganisms, and large multicellular organisms. Motility, coupled with various aspects of taxis, promotes rapid responses of bacteria to ambient changes and allows them to search actively for an optimal niche for habitation (Berg, 1975; Sauer, 2004; Flemming and Wingender, 2010; Guttenplan et al., 2013).

Planktonic bacteria move forward under the effect of the pushing force of their rotating flagella. When the direction of rotation of the organelles is reversed, a twitching force develops that makes bacteria move backward or somersault (Taylor and Koshland, 1974; Berg, 1975; Lele et al., 2013). This occurs repeatedly (the orientation of the cell changes by different angles), and, as a result, the trajectory of the cell movement becomes a broken line. Rotation of the flagellum causes rotation of the cell (Taylor and Kosh-

land, 1974; Berg, 1975; Chawla et al., 2020), but in the opposite direction. However, the cell makes fewer turns, because it is much larger than the flagellum. Thus, motile bacteria in planktonic culture experience a certain mechanical load of the environment, which may change as a result of hydrodynamic shear or alterations in the ambient mechanical parameters. An increase in the viscosity/density of the medium increases the load on the flagellum (Chawla et al., 2020), which affects the rate of cell movement and can influence both the morphology of bacteria and their behavior (McCarter, 1999; Whitchurch et al., 2004; Belas and Suvanasuthi, 2005; Harshey et al., 2015; Petrova et al., 2020).

In the case of viscous/gel-like/dense media and surfaces, bacteria switch from free swimming to swarming, i.e., to coordinated movement on wet surfaces, which depends on the functioning of motility organelles (flagella), intercellular contacts, production of surfactants, and some other factors (Harshey et al., 2015). Different bacterial species, including the pathogens *Vibrio parahaemolyticus* and *Proteus mirabilis* and the soil bacteria *Azospirillum brasilense* and *Azospirillum baldaniorum*, use swarming to colonize surfaces (Harshey et al., 2015). To implement this kind of motility, their cells increase in size and synthesize numerous additional flagella (McCarter et al., 1988; Kawagishi et al., 1996; Moens et al., 1996; Scheludko et al., 1998; Belas and Suvanasuthi, 2005; Petrova et al., 2020).

In deep-sea marine bacteria, flagellar motility responds to pressure changes (Eloe et al., 2008; Wang, F. et al., 2008). In the depths of the ocean, the phenotype of swarming bacteria may be preferable to the phenotype of planktonic cells (Dufrêne and Persat, 2020). Depending on the pressure, planktonic cells of these microbes convert to a swarming phenotype. The deep-sea bacteria *Photobacterium profundum* and *Shewanella piezotolerans*, growing optimally at high pressure, remain motile under pressure a thousand times higher than atmospheric pressure (Dufrêne and Persat, 2020), which is uncommon for *E. coli* and other microbes that are unable to adapt to these conditions (Eloe et al., 2008). The genome of the piezophile *Ph. profundum* contains two clusters of flagellar genes: one cluster encodes the polar flagellum, and the other putative cluster encodes the system of lateral flagella (Eloe et al., 2008). Lateral flagella are synthesized under high pressure or in highly viscous media to provide motility under these conditions (Eloe et al., 2008). Activation of the genes encoding the lateral flagellar system depends on the presence of the polar flagellum, which does not function at high pressure. These observations made it possible to conclude that *Ph. profundum* cells sense changes in pressure and viscosity by their polar flagellum and respond by activating the synthesis of lateral flagella, ensuring motility at high pressure (Dufrêne and Persat, 2020). Similarly, *Sh. piezotolerans* initiates swarming when pressure increases (Wang et al., 2008; Dufrêne and Persat, 2020).

The swimming planktonic bacteria *Pseudomonas aeruginosa* can radically change the way of their movement and switch to twitching motility owing to the mechanical work of type IV pili on viscous/gel-like surfaces (Whitchurch et al., 2004; McCallum et al., 2017). Motility organelles can also ensure physical contact of microbes with various surfaces, including other members of the ecological community, by acting as adhesins (Croes et al., 1993; Shelud'ko et al., 2010).

Motility and flagella facilitate the entry of bacteria into the interphase suitable for biofilm formation (solid/liquid or liquid/gas interface), penetration into an existing biofilm, and biofilm propagation over the surface (Houry et al., 2010). Bacterial biofilms are spatially and metabolically structured communities of microorganisms enclosed in a matrix consisting mainly of polysaccharides (PSs), proteins, and extracellular DNA (Flemming and Wingender, 2010). Bacterial flagella and pili are also integrated into the matrix and support its architecture, which depends on diverse factors, including hydrodynamic conditions, nutrient concentration, and bacterial motility and their communication with each other. During biofilm formation by bacteria differing in physiology and in the nature of interactions with the colonized object, general stages were identified. These are cell adhesion to the surface and the formation of microcolonies, monolayers, and multilayer biofilms. With aging, bio-

films undergo dispersion, and, as a result, bacteria switch to a planktonic lifestyle and search for new habitats (Verstraeten et al., 2008; López et al., 2010; Flemming and Wingender, 2010; Guttenplan et al., 2013). Biofilm decomposition can occur in three ways: erosion, sloughing, and dispersal of the cells (Kaplan, 2004). This stage is very important, because it results in the emergence of new free-living cells capable of forming a new biofilm, rather than in the death of biofilm cells (Sauer, 2004). Dispersal is most often a response to ambient changes (hydrodynamic shift, cessation of nutrient supply, or, conversely, the sudden supply of nutrients) (Sauer, 2004; Shelud'ko et al., 2019).

The diversity of regulatory formation mechanisms and biofilm structure elements is comparable to the number of species and even strains of bacteria that form these biofilms. Different strains of the same species often have different arsenals of signals and pathways that are important for the implementation of a particular behavioral response (López et al., 2010; Flemming and Wingender, 2010; Bogino et al., 2013).

Thus, bacteria are subject to diverse mechanical stimuli. Little is known about the perception of such effects, the transmission of signals about them, and the corresponding individual or group bacterial responses (changes in motility, transition from free swimming to swarming, adhesion to a surface, formation of colonies and biofilms, etc.) (Ellison and Brun, 2015; Persat, 2017; Chawla et al., 2020). There are examples of participation in these processes of outer membrane proteins and extracellular organelles (type I and IV pili and flagella), which are the first to make contact with the microenvironment of bacteria (Otto and Silhavy, 2002; Kuchma et al., 2012; Cairns et al., 2013; Belas, 2014; Blanka et al., 2015; Harapanahalli et al., 2015; Luo et al., 2015; Persat et al., 2015; Rodesney et al., 2017).

The molecular mechanisms of mechanosensitivity have been relatively well studied only in eukaryotes (Iskratsch et al., 2014; Ohashi et al., 2017; Fajardo-Cavazos and Nicholson, 2021). Therefore, obtaining the most complete information about the structures and molecular events that ensure the mechanical responses of bacteria is the task of current importance.

MECHANOSENSITIVITY OF THE CELL ENVELOPE

When bacteria are attached to a solid surface, they experience an adhesion force. This force causes a mechanical stress, which results in a reversible deformation of the cell membrane (Chen et al., 2014). The activity of bacterial mechanosensitive membrane channels, which open during the deformation of the lipid bilayer in the cell membrane, promotes osmoregulation (Sukharev et al., 1999; Booth, 2014). In some bacteria, membrane proteins sensitive to the deforma-

tion of the cell wall are part of the sensory–regulatory systems that transmit mechanical signals to the genetic apparatus of the cell (Otto and Silhavy, 2002).

In *Escherichia coli*, the CpxA/CpxR two-component sensory–regulatory system perceives various membrane perturbations. After receiving a signal from the periplasm, histidine kinase (CpxA) phosphorylates the cytoplasmic response regulator (CpxR), which includes the transcription of the controlled genes (Ruiz and Silhavy, 2005). Attachment to a hydrophobic surface activates CpxR-dependent gene transcription (Otto and Silhavy, 2002). Mutants in the *cpxA* gene are insensitive to contacts; therefore, the CpxA histidine kinase can perceive a signal that is induced when bacteria attach to the surface. For the corresponding activation of the Cpx system, the NlpE protein is required. It has been suggested that NlpE may be a surface sensor and that the state of this protein is “read” by the Cpx system. It is unclear how the adhesion force affects NlpE and activates CpxA-mediated signaling. Possibly, the contact with the surface induces changes in the conformation of NlpE and CpxA perceives these changes as the appearance of a misfolded protein (Otto and Silhavy, 2002).

The role of the CpxA/CpxR sensory–regulatory system in the perception of the signal induced by bacterial attachment to the surface was disputed (Kimkes and Heinemann, 2018). Those authors failed to detect the activation of the Cpx system during the contact of bacteria with a surface both at the cellular level and at the level of the population. However, when this research is compared to other studies (Otto and Silhavy, 2002; Shimizu et al., 2016), some methodological differences may be noted that complicate the final interpretation of the role of NlpE–CpxA/CpxR in surface sensing. Conflicting conclusions (Otto and Silhavy, 2002; Shimizu et al., 2016; Kimkes and Heinemann, 2018) indicate the need for further study of the role of NlpE–CpxA/CpxR in bacterial responses to surface attachment.

The Cpx system was found in numerous gram-negative bacteria; apart from maintaining the state of periplasmic proteins, it regulates virulence and other phenotypic characteristics (Raivio, 2005; Vogt and Raivio, 2012). For instance, during the contact with hydrophobic surfaces of enterohemorrhagic (EHEC) *E. coli* strains, the NlpE–Cpx system regulates the functioning of the type III secretion system (Shimizu et al., 2016).

PILI-MEDIATED MECHANOSENSITIVITY

Mechanosensory functions may probably be performed by type I and IV pili. Type I heteropolymer pili mediate the adhesion of bacteria to substrates (Busch and Waksman, 2012). Type IV pili are long, thin organelles that alternately stretch and contract owing to the polymerization and depolymerization of the

pilin protein subunits. They determine the twitching motility of bacteria and the formation of their microcolonies on surfaces (McCallum et al., 2017). In *E. coli*, the FimH adhesive subunit of type I pili appears able to perceive the shear force, which leads to increased adhesion of bacteria to the surface (Thomas et al., 2002).

In *P. aeruginosa*, the PilY1 surface-associated adhesin is required for the biosynthesis of pili and for the attachment of bacteria to the surface of eukaryotic cells. Presumably, it also participates in the regulation of *Pseudomonas* swarming. In 2014, PilY1 was shown to function as a bacterial mechanical sensor. Together with the minor PilW and PilX pilins, PilY1 proved required for surface contact-induced virulence. *Pseudomonas* mutants in the genes encoding other pilins were defective in the synthesis of pili but retained virulence. This indicated the participation of PilY1, PilW, and PilX in virulence regulation, which was independent of pili synthesis. By using directed mutagenesis, the participation of PilY1 in the regulation of bacterial virulence has been proven, although it is not clear whether this regulation is due to mechanosensing (Siryporn et al., 2014).

The PilA subunits of the pilin protein are the main structural components of type IV pili. Polymerization and depolymerization of the PilA monomers are regulated, respectively, by the PilB and PilT motors (Burrows, 2012). An increase in the cAMP content induced by surface contact depends on the PilA pilin, which suggests the involvement of type IV pili in the perception of *P. aeruginosa* contact with the surface (Luo et al., 2015; Persat et al., 2015). Mutations in the PilB and PilT motors and attachment to less dense surfaces decreased the level of mechanical responses (Persat et al., 2015). Studies on isolated type IV pili showed that their filaments could change conformation under the effect of tension (Biais et al., 2010; Beaussart et al., 2014), which possibly triggers a cascade of bacterial responses to mechanical stress (Persat, 2017).

When *P. aeruginosa* makes contacts with surfaces, the Vfr transcription factor acts as a positive regulator of type III secretion systems and twitching motility, determined by pili, and as a negative regulator of swarming. Transcription of the gene encoding Vfr is activated by a secondary messenger, cyclic adenosine monophosphate (cAMP). Expression of the Vfr-dependent genes is increased in the colonies growing on solid surfaces and is suppressed in planktonic cells. By using a fluorescent transcription reporter, the activation of cAMP/Vfr-dependent responses during contact with a surface was shown to be a response to a mechanical stimulus. This response depends on the activity of the type IV pili motor and the transmission of signals via the Chp sensor system. The Chp system is homologous to the Che chemotaxis system of *E. coli*. The methyl-accepting protein PilJ, which is a sensor

of the ambient stimuli, controls the level of phosphorylation of the ChpA histidine kinase and the corresponding PilG response regulator (Wolfgang et al., 2003; Whitchurch et al., 2004; Michel et al., 2011; Luo et al., 2015; Persat et al., 2015).

PARTICIPATION OF FLAGELLA IN THE PERCEPTION OF MECHANICAL STIMULI

Bacterial flagella, the motor organelles that are larger than pili, also take part in the perception of mechanical stimuli. Bacterial flagella have long been a popular object of research for molecular biologists; nevertheless, interest in their study is only increasing. New facts about the structure and variety of functions of these organelles are still being discovered. For instance, flagellin and the native flagella of various bacterial species were recently found to have proteolytic activity (Eckhard et al., 2017).

Bacterial flagella are composed of three main parts: a basal body, hook, and filament, which are synthesized in this order. Three main groups of flagellar genes are sequentially expressed from promoters of different types: (1) genes for the transcriptional activators of flagellar genes; (2) genes for the components of the basal body (proteinaceous MS-, C-, P-, and L-rings, type III secretion systems, and a rod) and hook; and (3) filament cap protein genes and flagellin genes. In the best-studied members of the *Gammaproteobacteria*, *E. coli* and *Salmonella enterica* with peritrichial flagellation, approximately 50 regulatory and structural genes are required for the assembly and operation of flagella. The expression of flagellar genes and the process of flagellar assembly are under multi-level regulatory control that responds to various signals and has its specific features in different bacterial classes, genera, and species (Brutinel and Yahr, 2008; Chevance and Hughes, 2008; Smith and Hoover, 2009; Patrick and Kearns, 2012; Tsang and Hoover, 2014; Altegoer and Bange, 2015; Osterman et al., 2015).

The basal body, which serves as an anchor of the flagellum in the cell membrane, a motor, and an export machine, includes several ring protein complexes, a core, and a type III secretion system. This secretion system exports the required proteins through the central channel of the flagellum during its assembly (Minamino, 2014). The expression of the flagellar genes is coordinated with the activity of the type III secretion system. As a result, secreted substrates appear only at the right moment of the flagellar assembly, and transcription of earlier flagellar genes is suppressed (Brutinel and Yahr, 2008). The expression of late flagellar genes is suppressed until the basal body is completely assembled, which contributes to the conservation of energy resources (Brutinel and Yahr, 2008).

The rotation of bacterial flagella is ensured by a motor located at the base of the flagellum in the cytoplasmic membrane and, like all motors, consisting of a rotor and a stator (the stator is formed by Mot proteins). The motor is set in motion, depending on the organism and the type of flagellum, by the flow of hydrogen ions or sodium ions through the plasma membrane; ATP hydrolysis is not required for the rotation of bacterial flagella. The rotating part of the motor consists of a set of rings that permeate the cell membrane and contain several hundred molecules of more than ten different proteins. The motor can rotate in different directions, ensuring either the translational motion of the cell or its somersault and spatial reorientation. That the complex of flagellar stators can perceive mechanical stimuli to the flagellum and respond to them is supported by an increase in the number of stator units in response to an increase in the load on the flagellum (Lele et al., 2013).

Existing hypotheses on how the flagella mediate the response of bacteria to changes in the density of the medium suggest that some signals inhibit the operation of the flagellar motor.

Specifically, mutations in the *fliL*, *fliF*, and *fliG* genes in the pathogenic bacterium *Pr. mirabilis*, encoding the components of the basal body of the flagellum, result in the differentiation of its cells to a swarming phenotype in liquid media, which are unsuitable for swarming, and to the formation of superlong cells on a solid medium. Moreover, the virulence of the bacteria increases. The *fliL* gene is part of the *fliLMNOPQR* operon, which also includes proteins of the flagellar rotor switch and proteins of the export apparatus. On the basis of a detailed characterization of the *fliL* mutants of *Pr. mirabilis*, FliL was concluded to be required for the transition of the bacteria to swarming and for the expression of virulence genes. Presumably, a small (17–18 kDa) FliL protein with an unclear function is associated with the basal body. *Pr. mirabilis* was hypothesized to determine its localization in the environment or in the host by assessing the status of its flagellar motors, which (the status) controls the expression of the genes for swarming and virulence (Belas and Suvanasuthi, 2005).

Subsequent transcriptomic analysis of the *Pr. mirabilis fliL* mutant showed that almost all (2) and (3) class flagellar genes, as well as chemotaxis genes, are repressed in it. Additional data allowed the authors to suggest that the C-terminus of FliL is involved in the perception of mechanical stimuli and that the UmoA regulator of swarming cells is part of the signal relay leading to the master flagellar operon of *Proteus* (Cusick et al., 2012).

Subsequently, data were obtained on the possible involvement of FliL in the control of proton flux when the motor is under high-torque conditions. According to the authors, FliL may participate in this control through the interaction of FliL with one of the motor

proteins, possibly MotB. The authors hypothesized that bacteria can sense the change in the proton-motive force, membrane potential, or pH gradient if flagellar rotation is inhibited on contact with the surface. After that, this surface signal triggers the transition of bacteria to swarming through a signaling pathway that includes the UmoA, UmoD, and Rsc proteins and increases the expression of the master flagellar operon (Lee and Belas, 2015). In *Proteus* and some other bacteria, this is the flhDC master operon, which is absent in *Alphaproteobacteria*. Other studies have shown that the described model of participation of the FliL protein in ensuring bacterial mechanical response does not apply to all bacteria (Chawla et al., 2017).

A study of mutants of the gram-positive bacterium *Bacillus subtilis* in the *motB* flagellar stator gene also revealed a possible mechanosensory role of flagella. Inhibition of flagellar motility by binding to polyclonal antibodies, as well as deletion of the *motB* gene, triggered biofilm formation. The mechanical response of *B. subtilis* depends on the DegS/DegU sensory-regulatory system. The cytoplasmic DegS sensory histidine kinase phosphorylates the DegU regulator in response to an external signal. The DegU phosphorylated response regulator activates the genes of the biofilm matrix components. Apparently, DegS perceives the signal generated on suppression of flagellar rotation by a presently unknown mechanism (Cairns et al., 2013).

A model was also proposed for the participation of the *Salmonella typhimurium* flagellum filament, together with the FlgM flagellar morphogenesis regulator, in the perception of external signals, in particular low humidity (and inhibition of filament growth in this case) (Wang et al., 2005). FlgM is transcribed from promoters of class (2) and, predominantly, class (3). In the first case, FlgM mainly remains in the cell; in the second case, it is secreted out of it. The authors suggested that the main purpose of excretion of the FlgM expressed from promoters of class (3) is to test whether the external conditions are favorable for flagella-mediated motility. This is especially important if surfaces have moisture levels that are critical for motility. The authors speculated that surface conditions favoring the facilitated secretion of FlgM may signal to cells that the number of flagellar filaments can be increased by transcription of late flagellar genes from class (3) promoters. In their model, developed from a study of swarming-defective *Salmonella* che mutants (although swarming does not depend on chemotaxis itself), the filament of the flagellum is the sensor of the surface properties (Wang et al., 2005).

Vibrio cholerae can also use its flagellum as a mechanosensory organelle. During the contact with a solid medium, arrest of the motor and cessation of the ion flow through the motor lead to an increase in the

membrane potential and to the initiation of biofilm formation (Van Dellen et al., 2008).

In *Vibrio parahaemolyticus*, a marine pathogenic bacterium with mixed flagellation, slowing down or blocking of the rotation of the polar flagellum triggers the assembly of lateral flagella, necessary for swarming over surfaces, and the expression of host colonization and virulence genes. Deletion of the *motB* flagellar stator gene or of the *flaC* flagellin gene also leads to constitutive transcription of the swarming genes, possibly as a result of a false “impression” of the mutant bacteria about the surface contact (McCarter et al., 1988; Kawagishi et al., 1996).

Like some other bacterial species, *A. brasilense* and *A. baldaniorum* can produce two types of flagella. The single polar flagellum (Fla) is produced constitutively, and numerous lateral flagella (Laf) are formed only at increased medium densities (for instance, in the presence of $\geq 0.4\%$ agar; Petrova et al., 2020). In liquids, *Azospirillum* bacteria swim rapidly owing to the functioning of Fla, whereas in viscous and semi-liquid media, they swarm. As in *A. brasilense* Sp7, the Fla of *A. baldaniorum* strain Sp245 (formerly *A. brasilense*; Dos Santos Ferreira et al., 2020) is covered with a polysaccharide sheath and the Fla flagellin is glycosylated (Moens et al., 1995; Burygin et al., 2007).

In some bacteria with mixed flagellation, the Laf system is possibly controlled by the Fla system (McCarter et al., 1988; Kawagishi et al., 1996; Moens et al., 1996). In *A. brasilense* strain Sp7, the expression of the *lafI* structural gene of the lateral flagellum flagellin was induced under conditions of hindered Fla rotation (on solid media or in liquids containing anti-flagellin polyclonal antibodies) (Moens et al., 1996). Yet, insertional nonswarming mutants of *A. baldaniorum* Sp245 and *Rhodospirillum centenum* SW (a bacterium in the same family *Rhodospirillaceae* as *Azospirillum*) were obtained that still produced inducible Laf at increased media densities, although their Fla was paralyzed or absent (Jiang et al., 1998; Scheludko et al., 1998). Thus, the signal used by azospirilla to induce Laf assembly appears more complicated than just difficulty in Fla rotation. Because the just-mentioned *Azospirillum* and *Rhodospirillum* mutants did not swarm, the functional Fla, possibly coordinating Laf and correct formation of the flagellar bundle, may also be necessary for *Azospirillum* and *R. centenum* swarming over the surface of a semi-liquid medium (Jiang et al., 1998; Scheludko et al., 1998; McClain et al., 2002).

In contrast to the nonswarming Fla[−] mutants of *A. baldaniorum* Sp245 and *R. centenum* SW, the Fla[−] mutants of *V. parahaemolyticus* and *Aeromonas hydrophila* (*Gammaproteobacteria*) retained their ability to swarm using Laf (McCarter et al., 1988). Moreover, when wild-type strains were transferred from liquid to solid media, the cells of *A. baldaniorum*, *A. brasilense*, *Aer. hydrophila*, and *Aer. caviae* elongated

slightly (Moens et al., 1995, 1996; Scheludko et al., 1998), as compared, for instance, to the cells of *V. parahaemolyticus*, which became several times longer (McCarter, 1999). Thus, the differentiation of the cells on surfaces and the nature of the interaction between the Fla and Laf systems have their distinctive features in different bacteria.

ROLE OF THE SECONDARY MESSENGER DIGUANOSINE MONOPHOSPHATE (c-di-GMP) IN BACTERIAL LIFESTYLE CHANGES

A secondary messenger such as cyclic diguanosine monophosphate (c-di-GMP) is crucial for the change in the lifestyle of bacteria and their switch, for instance, from planktonic to sessile or swarming states (Jenal, 2004; Roemling et al., 2005).

c-di-GMP is synthesized from two GTP molecules by bacterial diguanylate cyclases containing a GGDEF domain (Paul et al., 2004) and is hydrolyzed by phosphodiesterases characterized by the presence of an EAL or an HD-GYP domain (Christen et al., 2005; Ryan et al., 2006). The activity of these enzymes changes in response to external and internal signals, which results in changes in the cell concentration of c-di-GMP. This causes pronounced changes in the phenotype of bacteria, e.g., in their transition from solitary swimming to life in a multicellular biofilm (Jenal and Malone, 2006; Cotter and Stibitz, 2007; Monds et al., 2007; Newell et al., 2009; Hengge, 2009; Valentini and Filloux, 2016; Rodesney et al., 2017). However, little is known about how exactly the mechanical and other stimuli cause fluctuations in the concentration of c-di-GMP and other secondary messengers in the cells (Petrova and Sauer, 2012; Siryaporn et al., 2014; Luo et al., 2015). One model suggests that changes in the adhesion force that binds bacteria to the surface or in the rate of fluid flows that wash the surface-associated cells lead to changes in the cell concentration of c-di-GMP and, as a result, to the transition of bacteria from solitary swimming to existence in a multicellular biofilm (Rodesney et al., 2017). A high content of c-di-GMP reduces the synthesis and/or activity of flagella and stimulates the production of various bacterial adhesins and exopolysaccharides, which are components of the biofilm matrix (Hengge, 2009). A low content of cellular c-di-GMP is characteristic of planktonic free-swimming bacteria (Valentini and Filloux, 2016). By using model strains of *Pseudomonas aeruginosa*, *Vibrio cholerae*, and *Caulobacter crescentus*, data were obtained that prove the dependence of c-di-GMP synthesis on the activity of the flagellar motor (Hershey, 2021). The effect of mechanical forces on the activity of the flagellum or mutations in its motor genes cause responses that affect the production of c-di-GMP in bacteria (Hershey, 2021).

MECHANICAL RESPONSES

When bacteria experience mechanical loads, they form a mechanical response by modulating a variety of developmental and behavioral patterns. Contacts with surfaces mediate the regulation of bacterial virulence (Belas and Suvanasuthi, 2005; Raivio, 2005; Vogt and Raivio, 2012; Siryaporn et al., 2014) and the triggering of biofilm formation (Jenal and Malone, 2006; Cotter and Stibitz, 2007; Monds et al., 2007; Van Dellen et al., 2008; Newell et al., 2009; Hengge, 2009; Cairns et al., 2013; Valentini and Filloux, 2016; Rodesney et al., 2017). Changes in the cell size and in the mode of flagellation and the transition from free swimming to swarming occur in microbes that find their way from the liquid onto biotic/abiotic, viscous/gel-like or solid surfaces (Jiang et al., 1998; McCarter et al., 1988; Moens et al., 1995, 1996; Kawagishi et al., 1996; Scheludko et al., 1998; McCarter, 1999; McClain et al., 2002; Belas and Suvanasuthi, 2005; Bible et al., 2008; Eloë et al., 2008; Wang F. et al., 2008; Chawla et al., 2017; Petrova et al., 2020).

Individual or group responses of bacteria to changes in mechanical forces and ambient properties are not limited to the mechanical responses listed and briefly described in this review.

In the metal-reducing marine bacterium *Shewanella oneidensis*, flagella and type IV pili mediate mechanosensing (Dufrêne and Persat, 2020). Changes in the size (growth) and division of *She. oneidensis* cells that contact a surface differ from those observed in planktonic *She. oneidensis* cells (Dufrêne and Persat, 2020). The growth rates of surface-attached *She. oneidensis* mutants lacking flagella or type IV pili are similar to those of planktonic bacteria (Lee et al., 2016), which made it possible to point out an important role of these organelles in the perception of an ambient mechanical stimuli (Dufrêne and Persat, 2020). Deep-sea microorganisms encounter forces generated by high hydrostatic pressure. When moving from low-pressure to high-pressure growth conditions, deep-sea microorganisms undergo phenotypic changes that are not limited to the motility changes discussed above (Bartlett et al., 1989; Eloë et al., 2008; Wang F. et al., 2008). As the pressure increases, the protein and lipid composition of the outer membrane changes; the membrane becomes less fluid and more rigid (Bartlett et al., 1989).

The adhesive FimH subunit of *E. coli* type I pili perceives the force of hydrodynamic shear, which leads to increased adhesion of bacteria to the surface (Thomas et al., 2002). In the stalked bacterium *Caulobacter crescentus*, contact with the surface stimulates the synthesis of polysaccharides, which allow the cells to attach irreversibly to a solid substrate (Li et al., 2012). Pili and the rotating flagellum jointly mediate this response of *C. crescentus* (Li et al., 2012; Hug et al., 2017; Dufrêne and Persat, 2020). This process occurs in *Asticcacaulis biprosthecum*, *Agrobacterium*

tumefaciens, and *P. aeruginosa* in a similar way, which allows single planktonic cells to adapt rapidly to a sessile lifestyle and to biofilm formation (Dufrêne and Persat, 2020). *B. subtilis* responds to the surface attachment and inhibition of flagella rotation by activating the DegS–DegU two-component signaling system. The latter promotes transcription of the genes for the poly- γ -DL-glutamic acid exopolymer, which is a component of the biofilm matrix (Cairns et al., 2013). Some bacterial proteins have amyloid-forming amino acid sequences. Amyloids are found in the biofilms formed by bacteria of various taxonomic groups (Blanco et al., 2012). Amyloid structures form the framework of the films and mediate adhesion to surfaces or tissues, as well as the resistance of biofilms to various environmental factors (Larsen et al., 2007; Romero et al., 2010; Blanco et al., 2012). The effect of mechanical forces can promote the aggregation of amyloid adhesins in *E. coli* and *B. subtilis* (Dufrêne and Persat, 2020).

In biofilms, the matrix, which serves as a structural framework, is characterized by viscoelasticity (Flemming and Wingender, 2010; Douarche et al., 2015; Jana et al., 2020). The matrix exhibits opposite mechanical properties (viscous/elastic) in response to periodic or regular hydrodynamic shear forces and during the life cycle of biofilms (Jana et al., 2020). Bacteria in biofilms are genotypically, physiologically, and phenotypically heterogeneous (Stewart et al., 2008; Serra et al., 2014; Wang et al., 2017; Shelud'ko et al., 2020; Jana et al., 2020; Røder et al., 2020). The growth of bacteria can cause mechanical stress inside the biofilm, deforming the matrix, which creates mechanical stress and affects the orientation of cells in the community (Douarche et al., 2015). The relative localization of individual bacteria determines their interaction with each other, particularly in biofilms in which they are separated at a distance of $<1\ \mu\text{m}$ (Nadel et al., 2016; Tropini et al., 2017). For instance, spatial organization affects how individual cells perceive signaling molecules, such as autoinducers, nutrients, or antimicrobial factors (Mukherjee et al., 2019). In multispecies biofilms, the location of bacteria also influences interspecies social interactions, ultimately determining whether strains compete or cooperate (Nadel et al., 2016).

Environmental stress, such as hydrodynamic shear, which bacteria encounter during biofilm formation, can affect the properties of mature films, e.g., increase the elasticity and strength of the matrix proteins and polysaccharides (Herbert-Guillou et al., 2001; Lemos et al., 2015; Araujo et al., 2016). Apart from their effect on the transport of various signals and nutrients, mechanical forces created by fluid flows may damage the biofilm matrix, deliver new bacteria to biofilms, or promote the spread of cells from them, thus affecting the species composition of the population and the dynamics of bacterial species distribution (Kaplan, 2004; Sauer, 2004).

In this review, we have included only a small part of the extensive literature on the perception of mechanical stress by bacteria. A simple pattern of the microbial response to changes in the mechanical properties of the medium/environment includes a sequence of three elementary events: mechanotransmission, mechanosensitivity, and mechanical response (Iskratsch et al., 2014; Persat, 2017). Bacterial contact with a surface and/or hydrodynamic shear create(s) forces that are mechanically transmitted either by active organelles, such as flagella and type IV motile pili (which explore the environment mechanically), or by passive components such as the outer membrane, which becomes deformed under mechanical stress. Both active and passive organelles are associated with the sensory systems, which act as mechanosensitive components. This role may belong to chemosensory systems. Ultimately, the sequence of these events leads to mechanical responses. The c-di-GMP secondary messenger plays a certain role in changing the lifestyle of bacteria and their transition, for instance, from a planktonic to a sessile or swarming state (Jenal, 2004; Roemling et al., 2005). Noteworthy, mechanical responses are diverse. Bacterial cells change their morphology, modulate motility, activate virulence, or initiate biofilm formation. Such response changes in the bacterial phenotype, which are of interest to fundamental science, also have ecological, medical, agricultural, and biotechnological importance. Therefore, it would be promising to learn how to suppress the dangerous microbial mechanical responses or to stimulate the responses that are of practical use. The identification of mechanical stress as a signal leading to biofilm formation indicates the relevance of the approach to the design of surfaces that are not only bactericidal but also resist bacterial attachment or develop no mechanical stress (Salwiczek et al., 2014; MacCallum et al., 2015).

ACKNOWLEDGMENTS

The authors thank Prof. Dr. E.I. Katsy for developing the concept of literature data analysis and originating the research direction of bacterial mechanosensing and mechanotransduction.

FUNDING

This study was supported in part by the Russian Foundation for Basic Research (project no. 20-04-00006-a, “Genetic Aspects of Mechanosensitivity of *Alphaproteobacteria* with Mixed Flagellation in *Azospirillum brasilense*”).

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interests. The authors declare that they have no conflict of interest.

Statement on the welfare of animals. This article does not contain any studies involving animals or human participants performed by any of the authors.

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REFERENCES

- Altegoer, F. and Bange, G., Undiscovered regions on the molecular landscape of flagellar assembly, *Curr. Opin. Microbiol.*, 2015, vol. 28, pp. 98–105.
- Araújo, P.A., Malheiro, J., Machado, I., Mergulhão, F., Melo, L., and Simões, M., Influence of flow velocity on the characteristics of *Pseudomonas fluorescens* biofilms, *J. Environ. Eng.*, 2016, vol. 142, pp. 04016031-1–04016031-8.
- Bartlett, D., Wright, M., Yayanos, A.A., and Silverman, M., Isolation of a gene regulated by hydrostatic pressure in a deep-sea bacterium, *Nature*, 1989, vol. 342, pp. 572–574.
- Beaussart, A., Baker, A.E., Kuchma, S.L., El-Kirat-Chatel, S., O'Toole, G.A., and Dufrene, Y.F., Nanoscale adhesion forces of *Pseudomonas aeruginosa* type IV pili, *ACS Nano*, 2014, vol. 8, pp. 10723–10733.
- Belas, R., Biofilms, flagella, and mechanosensing of surfaces by bacteria, *Trends Microbiol.*, 2014, vol. 22, pp. 517–527.
- Belas, R. and Sivanasuthi, R., The ability of *Proteus mirabilis* to sense surfaces and regulate virulence gene expression involves FliL, a flagellar basal body protein, *J. Bacteriol.*, 2005, vol. 187, pp. 6789–6803.
- Berg, H.C., Bacterial behaviour, *Nature*, 1975, vol. 254, pp. 389–392.
- Biais, N., Higashi, D.L., Brujic, J., So, M., and Sheetz, M.P., Force-dependent polymorphism in type IV pili reveals hidden epitopes, *Proc. Natl. Acad. Sci. U. S. A.*, 2010, vol. 107, pp. 11358–11363.
- Bible, A.N., Stephens, B.B., Ortega, D.R., Xie, Z., and Alexandre, G., Function of a chemotaxis-like signal transduction pathway in modulating motility, cell clumping, and cell length in the alphaproteobacterium *Azospirillum brasilense*, *J. Bacteriol.*, 2008, vol. 190, pp. 6365–6375.
- Blanco, L.P., Evans, M.L., Smith, D.R., Badtke, M.P., and Chapman, M.R., Diversity, biogenesis and function of microbial amyloids, *Trends Microbiol.*, 2012, vol. 20, pp. 66–73.
- Blanka, A., Düvel, J., Dötsch, A., Klinkert, B., Abraham, W.-R., Kaever, V., Ritter, C., Narberhaus, F., and Häussler, S., Constitutive production of c-di-GMP is associated with mutations in a variant of *Pseudomonas aeruginosa* with altered membrane composition, *Sci. Signal*, 2015, vol. 8, p. ra36.
- Bogino, P.C., Oliva, M.M., Sorroche, F.G., and Giordano, W., The role of bacterial biofilms and surface components in plant-bacterial associations, *Int. J. Mol. Sci.*, 2013, vol. 14, pp. 15838–15859.
- Booth, I.R., Bacterial mechanosensitive channels: progress towards an understanding of their roles in cell physiology, *Curr. Opin. Microbiol.*, 2014, vol. 18, pp. 16–22.
- Brutinel, E.D. and Yahr, T.L., Control of gene expression by type III secretory activity, *Curr. Opin. Microbiol.*, 2008, vol. 11, pp. 128–133.
- Burrows, L.L., *Pseudomonas aeruginosa* twitching motility: type IV pili in action, *Annu. Rev. Microbiol.*, 2012, vol. 66, pp. 493–520.
- Burygin, G.L., Shirokov, A.A., Shelud'ko, A.V., Katsy, E.I., Shchygolev, S.Yu., and Matora, L.Yu. Detection of a sheath on *Azospirillum brasilense* polar flagellum, *Microbiology (Moscow)*, 2007, vol. 76, pp. 728–734.
- Busch, A. and Waksman, G., Chaperone-usher pathways: diversity and pilus assembly mechanism, *Philos. Trans. R. Soc. Lond. B Biol. Sci.*, 2012, vol. 367, pp. 1112–1122.
- Cairns, L.S., Marlow, V.L., Bissett, E., Ostrowski, A., and Stanley-Wall, N.R., A mechanical signal transmitted by the flagellum controls signalling in *Bacillus subtilis*, *Mol. Microbiol.*, 2013, vol. 90, pp. 6–21.
- Chawla, R., Ford, K.M., and Lele, P.P., Torque, but not FliL, regulates mechanosensitive flagellar motor-function, *Sci. Rep.*, 2017, vol. 7, art. 5565.
- Chawla, R., Gupta, R., Lele, T.P., and Lele, P.P. A skeptic's guide to bacterial mechanosensing, *J. Mol. Biol.*, 2020, vol. 432, pp. 523–533.
- Chen, Y., Harapanahalli, A.K., Busscher, H.J., Norde, W., and van der Mei, H.C., Nanoscale cell wall deformation impacts long-range bacterial adhesion forces on surfaces, *Appl. Environ. Microbiol.*, 2014, vol. 80, pp. 637–643.
- Chevance, F.F.V. and Hughes, K.T., Coordinating assembly of a bacterial macromolecular machine, *Nat. Rev. Microbiol.*, 2008, vol. 6, pp. 455–465.
- Christen, M., Christen, B., Folcher, M., Schauerte, A., and Jenal, U., Identification and characterization of a cyclic di-GMP-specific phosphodiesterase and its allosteric control by GTP, *J. Biol. Chem.*, 2005, vol. 280, pp. 30829–30837.
- Cotter, P.A. and Stibitz, S., c-di-GMP-mediated regulation of virulence and biofilm formation, *Curr. Opin. Microbiol.*, 2007, vol. 10, pp. 17–23.
- Croes, C.L., Moens, S., van Bastelaere, E., Vanderleyden, J., and Michiels, K.W., The polar flagellum mediates *Azospirillum brasilense* adsorption to wheat roots, *J. Gen. Microbiol.*, 1993, vol. 139, pp. 2261–2269.
- Cusick, K., Lee, Y.-Y., Youchak, B., and Belas, R., Perturbation of FliL interferes with *Proteus mirabilis* swarmer cell gene expression and differentiation, *J. Bacteriol.*, 2012, vol. 194, pp. 437–447.
- Dos Santos Ferreira, N., Hayashi Sant'Anna, F., Massena Reis, V., Ambrosini, A., Gazolla, Volpiano, C., Rothballer, M., Schwab, S., Baura, V.A., Balsanelli, E., Pedrosa, F.O., Pereira, Passaglia, L.M., Maltempi de

- Souza, E., Hartmann, A., et al., Genome-based reclassification of *Azospirillum brasilense* Sp245 as the type strain of *Azospirillum baldaniorum* sp. nov., *Int. J. Syst. Evol. Microbiol.*, 2020, vol. 70, pp. 6203–6212.
- Douarche, C., Allain, J.-M., and Raspaud, E., *Bacillus subtilis* bacteria generate an internal mechanical force within a biofilm, *Biophys. J.*, 2015, vol. 109, pp. 2195–2202.
- Dufrène, Y.F. and Persat, A., Mechanomicrobiology: how bacteria sense and respond to forces, *Nature Rev. Microbiol.*, 2020, vol. 18, pp. 227–240.
- Eckhard, U., Bandukwala, H., Mansfield, M.J., Marino, G., Cheng, J., Wallace, I., Holyoak, T., Charles, T.C., Austin, J., Overall, C.M., and Doxey, A.C., Discovery of a proteolytic flagellin family in diverse bacterial phyla that assembles enzymatically active flagella, *Nat. Commun.*, 2017, vol. 8, art. 521.
- Ellison, C. and Brun, Y.V., Mechanosensing: a regulation sensation, *Curr. Biol.*, 2015, vol. 25, pp. R113–R115.
- Eloe, E.A., Lauro, F.M., Vogel, R.F., and Bartlett, D.H., The deep-sea bacterium *Photobacterium profundum* ss9 utilizes separate flagellar systems for swimming and swarming under high-pressure conditions, *Appl. Environ. Microbiol.*, 2008, vol. 74, pp. 6298–6305.
- Fajardo-Cavazos, P. and Nicholson, W.L., Mechanotransduction in prokaryotes: a possible mechanism of spaceflight adaptation, *Life*, 2021, vol. 11, pp. 33–44.
- Fibach-Paldi, S., Burdman, S., and Okon, Y., Key physiological properties contributing to rhizosphere adaptation and plant growth promotion abilities of *Azospirillum brasilense*, *FEMS Microbiol. Lett.*, 2012, vol. 326, pp. 99–108.
- Flemming, H.-C. and Wingender, J., The biofilm matrix, *Nat. Rev. Microbiol.*, 2010, vol. 8, pp. 623–633.
- Guttenplan, S.B. and Kearns, D.B., Regulation of flagellar motility during biofilm formation, *FEMS Microbiol. Rev.*, 2013, vol. 37, pp. 849–871.
- Harapanahalli, A.K., Younes, J.A., Allan, E., van der Mei, H.C., and Busscher, H.J., Chemical signals and mechanosensing in bacterial responses to their environment, *PLoS Pathog.*, 2015, vol. 11, pp. e1005057 (1–6).
- Harshey, R.M. and Partridge, J.D., Shelter in a swarm, *J. Mol. Biol.*, 2015, vol. 427, pp. 3683–3694.
- Hengge, R., Principles of c-di-GMP signalling in bacteria, *Nature Rev. Microbiol.*, 2009, vol. 7, pp. 263–273.
- Herbert-Guillou, D., Tribollet, B., and Festy, D., Influence of the hydrodynamics on the biofilm formation by mass transport analysis, *Bioelectrochem.*, 2001, vol. 53, pp. 119–125.
- Hershey, D.M., Integrated control of surface adaptation by the bacterial flagellum, *Curr. Opin. Microbiol.*, 2021, vol. 61, pp. 1–7.
- Houry, A., Briandet, R., Aymerich, S., and Gohar, M., Involvement of motility and flagella in *Bacillus cereus* biofilm formation, *Microbiology (SGM)*, 2010, vol. 156, pp. 1009–1018.
- Hug, I., Deshpande, S., Sprecher, K.S., Pfohl, T., and Jenal, U., Second messenger-mediated tactile response by a bacterial rotary motor, *Science*, 2017, vol. 358, pp. 531–534.
- Iskratsch, T., Wolfenson, H., and Sheetz, M.P., Appreciating force and shape—the rise of mechanotransduction in cell biology, *Nat. Rev. Mol. Cell Biol.*, 2014, vol. 15, pp. 825–833.
- Jana, S., Charlton, S.G.V., Eland, L.E., Burgess, J.G., Wipat, A., Curtis, T.P., and Chen, J., Nonlinear rheological characteristics of single species bacterial biofilms, *NPJ Biofilms Microbiomes.*, 2020, vol. 6, art. 19. <https://doi.org/10.1038/s41522-020-0126-1>
- Jenal, U., Cyclic di-guanosine-monophosphate comes of age: a novel secondary messenger involved in modulating cell surface structures in bacteria?, *Curr. Opin. Microbiol.*, 2004, vol. 7, pp. 185–191.
- Jenal, U. and Malone, J., Mechanisms of cyclic-di-GMP signaling in bacteria, *Annu. Rev. Genet.*, 2006, vol. 40, pp. 385–407.
- Jiang, Z.Y., Rushing, B.G., Bai, Y., Gest, H., and Bauer, C.E., Isolation of *Rhodospirillum centenum* mutants defective in phototactic colony motility by transposon mutagenesis, *J. Bacteriol.*, 1998, vol. 180, pp. 1248–1255.
- Kaplan, J.B., Velliyagounder, K., Ragunath, C., Rohde, H., Mack, D., Knobloch, J.K., and Ramasubbu, N., Genes involved in the synthesis and degradation of matrix polysaccharide in *Actinobacillus actinomycetemcomitans* and *Actinobacillus pleuropneumoniae* biofilms, *J. Bacteriol.*, 2004, vol. 186, pp. 8213–8220.
- Kawagishi, I., Imagawa, M., Imae, Y., McCarter, L., and Homma, M., The sodium-driven polar flagellar motor of marine *Vibrio* as the mechanosensor that regulates lateral flagellar expression, *Mol. Microbiol.*, 1996, vol. 20, pp. 693–699.
- Kimkes, T.E.P. and Heinemann, M., Reassessing the role of the *Escherichia coli* CpxAR system in sensing surface contact, *PLoS One*, 2018, vol. 13, p. e0207181.
- Kuchma, S.L., Griffin, E.F., and O'Toole, G.A., Minor pilins of the type IV pilus system participate in the negative regulation of swarming motility, *J. Bacteriol.*, 2012, vol. 194, pp. 5388–5403.
- Larsen, P., Nielsen, J.L., Dueholm, M.S., Wetzel, R., Otzen, D., and Nielsen, P.H., Amyloid adhesins are abundant in natural biofilms, *Environ. Microbiol.*, 2007, vol. 9, pp. 3077–3090.
- Lee, C.K., Kim, A.J., Santos, G.S., Lai, P.Y., Lee, S.Y., Qiao, D.F., Anda, J.D., Young, T.D., Chen, Y., Rowe, A.R., Nealson, K.H., Weiss, P.S., and Wong, G.C.L., Evolution of cell size homeostasis and growth rate diversity during initial surface colonization of *Shewanella oneidensis*, *ACS Nano*, 2016, vol. 10, pp. 9183–9192.
- Lee, Y.-Y. and Belas, R., Loss of FlhL alters *Proteus mirabilis* surface sensing and temperature-dependent swarming, *J. Bacteriol.*, 2015, vol. 197, pp. 159–173.
- Lele, P.P., Hosu, B.G., and Berg, H.C., Dynamics of mechanosensing in the bacterial flagellar motor, *Proc. Natl. Acad. Sci. U. S. A.*, 2013, vol. 110, pp. 11839–11844.
- Lemos, M., Mergulhao, F., Melo, L., and Simoes, M., The effect of shear stress on the formation and removal of *Bacillus cereus* biofilms, *Food Bioprod. Process.*, 2015, vol. 93, pp. 242–248.
- Li, G., Brown, P.J.B., Tang, J.X., Xu, J., Quardokus, E.M., Fuqua, C., and Brun, Y.V., Surface contact stimulates the just-in-time deployment of bacterial adhesins, *Mol. Microbiol.*, 2012, vol. 83, pp. 41–51.
- López, D., Vlamakis, H., and Kolter, R., *Biofilms*, Cold Spring Harb. Perspect. Biol. 2010, vol. 2, p. a000398 (1–11).

- Luo, Y., Zhao, K., Baker, A.E., Kuchma, S.L., Coggan, K.A., Wolfgang, M.C., Wong, G.C., and O'Toole, G.A., A hierarchical cascade of second messengers regulates *Pseudomonas aeruginosa* surface behaviors, *mBio*, 2015, vol. 6, p. e02456-14 (1–11).
- MacCallum, N., Howell, C., Kim, P., Sun, D., Friedlander, R., Ranisau, J., Ahanotu, O., Lin, J.J., Vena, A., Hutton, B., Wong, T.-S., and Aizenberg, J., Liquid-infused silicone as a biofouling-free medical material, *ACS Biomater. Sci. Eng.*, 2015, vol. 1, pp. 43–51.
- McCallum, M., Tammam, S., Khan, A., Burrows, L.L., and Howell, P.L., The molecular mechanism of the type IVa pilus motors, *Nat. Commun.*, 2017, vol. 8, p. 15091 (1–10).
- McCarter, L., Hilmen, M., and Silverman, M., Flagellar dynamometer controls swarmer cell differentiation of *V. parahaemolyticus*, *Cell*, 1988, vol. 54, pp. 345–351.
- McCarter, L., The multiple identities of *Vibrio parahaemolyticus*, *J. Mol. Microbiol. Biotechnol.*, 1999, vol. 1, pp. 51–57.
- McClain, J., Rollo, D.R., Rushing, B.G., and Bauer, C.E., *Rhodospirillum centenum* utilizes separate motor and switch components to control lateral and polar flagellum rotation, *J. Bacteriol.*, 2002, vol. 184, pp. 2429–2438.
- Michel, G.P.F., Aguzzi, A., Ball, G., Soscia, C., Bleves, S., and Voulhoux, R., Role of *fimV* in type II secretion system-dependent protein secretion of *Pseudomonas aeruginosa* on solid medium, *Microbiology (SGM)*, 2011, vol. 157, no. 7, pp. 1945–1954.
- Minamino, T., Protein export through the bacterial flagellar type III export pathway, *Biochim. Biophys. Acta*, 2014, vol. 1843, pp. 1642–1648.
- Moens, S., Michiels, K., and Vanderleyden, J., Glycosylation of the flagellin of the polar flagellum of *Azospirillum brasilense*, a Gram-negative nitrogen-fixing bacterium, *Microbiology (SGM)*, 1995, vol. 141, pp. 2651–2657.
- Moens, S., Schlöter, M., and Vanderleyden, J., Expression of the structural gene, *lafI*, encoding the flagellin of the lateral flagella in *Azospirillum brasilense* Sp7, *J. Bacteriol.*, 1996, vol. 178, pp. 5017–5019.
- Monds, R.D., Newell, P.D., Gross, R.H., and O'Toole, G.A., Phosphate-dependent modulation of c-di-GMP levels regulates *Pseudomonas fluorescens* Pf0-1 biofilm formation by controlling secretion of the adhesin LapA, *Mol. Microbiol.*, 2007, vol. 63, pp. 656–679.
- Mukherjee, S. and Bassler, B.L., Bacterial quorum sensing in complex and dynamically changing environments, *Nat. Rev. Microbiol.*, 2019, vol. 17, pp. 371–382.
- Nadell, C.D., Drescher, K., and Foster, K.R., Spatial structure, cooperation and competition in biofilms, *Nat. Rev. Microbiol.*, 2016, vol. 14, pp. 589–600.
- Newell, P.D., Monds, R.D., and O'Toole, G.A., LapD is a bis-(3',5')-cyclic dimeric GMP-binding protein that regulates surface attachment by *Pseudomonas fluorescens* Pf0-1, *Proc. Natl. Acad. Sci. U. S. A.*, 2009, vol. 106, pp. 3461–3466.
- Ohashi, K., Fujiwara, S., and Mizuno, K., Roles of the cytoskeleton, cell adhesion and rho signalling in mechanosensing and mechanotransduction, *J. Biochem.*, 2017, vol. 161, pp. 245–254.
- Osterman, I.A., Dikhtyar, Y.Y., Bogdanov, A.A., Dontsova, O.A., and Sergiev, P.V., Regulation of flagellar gene expression in bacteria, *Biochemistry (Moscow)*, 2015, vol. 80, pp. 1447–1456.
- Otto K., Silhavy T.J., Surface sensing and adhesion of *Escherichia coli* controlled by the Cpx-signaling pathway, *Proc. Natl. Acad. Sci. U. S. A.*, 2002, vol. 99, pp. 2287–2292.
- Patrick, J.E. and Kearns, D.B., Swarming motility and the control of master regulators of flagellar biosynthesis, *Mol. Microbiol.*, 2012, vol. 83, pp. 14–23.
- Paul, R., Weiser, S., Amiot, N.C., Chan, C., Schirmer, T., Giese, B., and Jenal, U., Cell cycle-dependent dynamic localization of a bacterial response regulator with a novel diguanylate cyclase output domain, *Genes Dev.*, 2004, vol. 18, pp. 715–727.
- Persat, A., Bacterial mechanotransduction, *Curr. Opin. Microbiol.*, 2017, vol. 36, pp. 1–6.
- Persat, A., Inclan, Y.F., Engel, J.N., Stone, H.A., and Gital, Z., Type IV pili mechanoschemically regulate virulence factors in *Pseudomonas aeruginosa*, *Proc. Natl. Acad. Sci. U. S. A.*, 2015, vol. 112, pp. 7563–7568.
- Petrova, L.P., Yevstigneyeva, S.S., Borisov, I.V., Shelud'ko, A.V., Burygin, G.L., and Katsy E.I., Plasmid gene AZOBR_p60126 impacts biosynthesis of lipopolysaccharide II and swarming motility in *Azospirillum brasilense* Sp245, *J. Basic Microbiol.*, 2020, vol. 60, pp. 613–623.
- Petrova, O.E. and Sauer, K., Sticky situations: key components that control bacterial surface attachment, *J. Bacteriol.*, 2012, vol. 194, pp. 2413–2425.
- Raivio, T.L., Envelope stress responses and Gram-negative bacterial pathogenesis, *Mol. Microbiol.*, 2005, vol. 56, pp. 1119–1128.
- Røder, H.L., Olsen, N.M.C., Whiteley, M., and Burmølle, M., Unravelling interspecies interactions across heterogeneities in complex biofilm communities, *Environ. Microbiol.*, 2020, vol. 22, pp. 5–16.
- Rodesney, C.A., Roman, B., Dhamani, N., Cooley, B.J., Katira, P., Touhami, A., and Gordon, V.D., Mechanosensing of shear by *Pseudomonas aeruginosa* leads to increased levels of the cyclic-di-GMP signal initiating biofilm development, *Proc. Natl. Acad. Sci. U. S. A.*, 2017, vol. 114, pp. 5906–5911.
- Romero, D., Aguilar, C., Losick, R., and Kolter R., Amyloid fibers provide structural integrity to *Bacillus subtilis* biofilms, *Proc. Natl. Acad. Sci. U. S. A.*, 2010, vol. 107, pp. 2230–2234.
- Römling, U., Gomelsky, M., and Galperin, M.Y., C-di-GMP: the dawning of a novel bacterial signalling system, *Mol. Microbiol.*, 2005, vol. 57, pp. 629–639.
- Ruiz, N. and Silhavy, T.J., Sensing external stress: watchdogs of the *Escherichia coli* cell envelope, *Curr. Opin. Microbiol.*, 2005, vol. 8, pp. 122–126.
- Ryan, R.P., Fouhy, Y., Lucey, J.F., Crossman, L.C., Spiro, S., He, Y.-W., Zhang, L.-H., Heeb, S., Cámara, M., Williams, P., and Dow, J.M., Cell-cell signaling in *Xanthomonas campestris* involves an HD-GYP domain protein that functions in cyclic di-GMP turnover, *Proc. Natl. Acad. Sci. U. S. A.*, 2006, vol. 103, pp. 6712–6717.
- Salwiczek, M., Qu, Y., Gardiner, J., Strugnelli, R.A., Lithgow, T., McLean, K.M., and Thissen, H., Emerging rules for effective antimicrobial coatings, *Trends Biotechnol.*, 2014, vol. 32, pp. 82–90.
- Sauer, K., Cullen, M.C., Rickard, A.H., Zeef, L.A.H., Davies, D.G., and Gilbert, P., Characterization of nutrient-

- induced dispersion in *Pseudomonas aeruginosa* PAO1 biofilm, *J. Bacteriol.*, 2004, vol. 186, pp. 7312–7326.
- Schelud'ko, A.V., Katsy, E.I., Ostudin, N.A., Gringauz, O.K., and Panasenkov, V.I., Novel classes of *Azospirillum brasilense* mutants with defects in the assembly and functioning of polar and lateral flagella, *Mol. Gen. Mikrobiol. Virusol.*, 1998, no. 4, pp. 33–37.
- Serra, D.O. and Hengge, R., Stress responses go three dimensional—the spatial order of physiological differentiation in bacterial macrocolony biofilms, *Environ. Microbiol.*, 2014, vol. 16, pp. 1455–1471.
- Shelud'ko, A.V., Filip'echeva, Yu.A., Telesheva, E.M., Yevstigneeva, S.S., Petrova, L.P., and Katsy, E.I., Polar flagellum of the alphaproteobacterium *Azospirillum brasilense* Sp245 plays a role in biofilm biomass accumulation and in biofilm maintenance under stationary and dynamic conditions, *World J. Microbiol. Biotechnol.*, 2019, vol. 35, p. 19.
- Shelud'ko, A.V., Mokeev, D.I., Evstigneeva, S.S., Filip'echeva, Yu.A., Burov, A.M., Petrova, L.P., Ponomareva, E.G., and Katsy, E.I., Cell ultrastructure in biofilms of *Azospirillum brasilense*, *Microbiology (Moscow)*, 2020, vol. 89, pp. 50–63.
- Shelud'ko, A.V., Shirokov, A.A., Sokolova, M.K., Sokolov, O.I., Petrova, L.P., Matora, L.Y., and Katsy, E.I., Wheat root colonization by *Azospirillum brasilense* strains with different motility, *Microbiology (Moscow)*, 2010, vol. 79, pp. 688–695.
- Shimizu, T., Ichimura, K., and Noda, M., The surface sensor NlpE of enterohemorrhagic *Escherichia coli* contributes to regulation of the type III secretion system and flagella by the Cpx response to adhesion, *Infect. Immun.*, 2015, vol. 84, pp. 537–549.
- Siryaporn, A., Kuchma, S.L., O'Toole, G.A., and Gitai, Z., Surface attachment induces *Pseudomonas aeruginosa* virulence, *Proc. Natl. Acad. Sci. U. S. A.*, 2014, vol. 111, pp. 16860–16865.
- Smith, T.G. and Hoover, T.R., Deciphering bacterial flagellar gene regulatory networks in the genomic era, *Adv. Appl. Microbiol.*, 2009, vol. 67, pp. 257–295.
- Stewart, P.S. and Franklin, M.J., Physiological heterogeneity in biofilms, *Nat. Rev. Microbiol.*, 2008, vol. 6, pp. 199–210.
- Sukharev, S.I., Sigurdson, W.J., Kung, C., and Sachs, F., Energetic and spatial parameters for gating of the bacterial large conductance mechanosensitive channel, MscL, *J. Gen. Physiol.*, 1999, vol. 113, pp. 525–540.
- Taylor, B.L. and Koshland Jr., D.E., Reversal of flagellar rotation in monotrichous and peritrichous bacteria: generation of changes in direction, *J. Bacteriol.*, 1974, vol. 119, pp. 640–642.
- Thomas, W.E., Trintchina, E., Forero, M., Vogel, V., and Sokurenko, E.V., Bacterial adhesion to target cells enhanced by shear force, *Cell*, 2002, vol. 109, pp. 913–923.
- Tropini, C., Earle, K.A., Huang, K.C., and Sonnenburg, J.L., The gut microbiome: connecting spatial organization to function, *Cell Host Microbe*, 2017, vol. 21, pp. 433–442.
- Tsang, J. and Hoover, T.R., Themes and variations: regulation of RpoN-dependent flagellar genes across diverse bacterial species, *Scientifica*, 2014, vol. 2014, art. 681754 (1–14).
- Valentini, M. and Filloux, A., Biofilms and cyclic di-GMP (c-di-GMP) signaling: lessons from *Pseudomonas aeruginosa* and other bacteria, *J. Biol. Chem.*, 2016, vol. 291, pp. 12547–12555.
- Van Dellen, K.L., Houot, L., and Watnick, P.I., Genetic analysis of *Vibrio cholerae* monolayer formation reveals a key role for DeltaPsi in the transition to permanent attachment, *J. Bacteriol.*, 2008, vol. 190, pp. 8185–8196.
- Verstraeten, N., Braeken, K., Debkumari, B., Fauvart, M., Franssaer, J., Vermant, J., and Michiels, J., Living on a surface: swarming and biofilm formation, *Trends Microbiol.*, 2008, vol. 16, pp. 496–506.
- Vladimirov, N. and Sourjik, V., Chemotaxis: how bacteria use memory, *Biol. Chem.*, 2009, vol. 390, pp. 1097–2104.
- Vogt, S.L. and Raivio, T.L., Just scratching the surface: an expanding view of the Cpx envelope stress response, *FEMS Microbiol. Lett.*, 2012, vol. 326, pp. 2–11.
- Wang, D., Xu, A., Elmerich, C., and Ma, L.Z., Biofilm formation enables free-living nitrogen-fixing rhizobacteria to fix nitrogen under aerobic conditions, *ISME J.*, 2017, vol. 11, pp. 1602–1613.
- Wang, F., Wang, J., Jian, H., Zhang, B., Li, S., Wang, F., Zeng, X., Gao, L., Bartlett, D.H., Yu, J., Hu, S., and Xiao, X., Environmental adaptation: genomic analysis of the piezotolerant and psychrotolerant deep-sea iron reducing bacterium *Shewanella piezotolerans* WP3, *PLoS One*, 2008, vol. 3, p. e1937.
- Wang, Q., Suzuki, A., Mariconda, S., Porwollik, S., and Harshey, R.M., Sensing wetness: a new role for the bacterial flagellum, *EMBO J.*, 2005, vol. 24, pp. 2034–2042.
- Whitchurch, C.B., Leech, A.J., Young, M.D., Kennedy, D., Sargent, J.L., Bertrand, J.J., Semmler, A.B., Mellick, A.S., Martin, P.R., Alm, R.A., Hobbs, M., Beatson, S.A., Huang, B., Nguyen, L., Commolli, J.C., et al., Characterization of a complex chemosensory signal transduction system which controls twitching motility in *Pseudomonas aeruginosa*, *Mol. Microbiol.*, 2004, vol. 52, pp. 873–893.
- Wolfgang, M.C., Lee, V.T., Gilmore, M.E., and Lory, S., Coordinate regulation of bacterial virulence genes by a novel adenylate cyclase-dependent signaling pathway, *Dev. Cell*, 2003, vol. 4, pp. 253–263.

Translated by A. Panyushkina